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OPHTHALMOLOGY

PART II

Symposium on Visual Mechanism

Held at the National Institutes of Health

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TABLE OF CONTENTS

VOLUME 60 OCTOBER 1958 NUMBER 4

Part II

PAGE

Welcome
Phillips Thygeson, M.D., San Francisco 687

The Lipoprotein Nature of Rhodopsin
Norman I. Krinsky, Cambridge, Mass. 688

The Behavior of Flash-Illuminated Rhodopsin in Solution
Verner J. Wulff, Ralph G. Adams, Henry Linschitz, and Donald Kennedy, Syracuse, N. Y. 695

Differentiation, Metabolic Organization, and Viability of the Visual Cell
Werner K. Noell, M.D., Buffalo 702

Inhibitory Interaction in the Limulus Eye—Abstract
H. K. Hartline, New York 734

The Human Electroretinogram
Lorrin A. Riggs, Providence, R. I. 739

Present Status of Our Knowledge of Stereoscopic Vision
Kenneth N. Ogle, Ph.D., Rochester, Minn. 755

Some Facts and Concepts Regarding the Neurophysiology of the Optic Pathway
S. Howard Bartley, East Lansing, Mich. 775

Color Blindness and Color Theory
C. H. Graham and Yun Hsia, New York 792

On-Responses in the Human Visual System as Inferred from Psychophysical Studies of Rapid-Adaptation
Robert M. Boynton, Ph.D., Rochester, N. Y. 800

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A.M.A. ARCHIVES OF
OPHTHALMOLOGY

Welcome

PHILLIPS THYGESON, M.D., San Francisco

On behalf of the Section I am very happy to welcome you to this symposium. We have all been looking forward to it eagerly.

You may be interested to know that this is the third meeting of this kind that the Study Section has held. The first one, under the chairmanship of Dr. Jonas Friedenwald, was for the purpose of considering retrolental fibroplasia. It was a joint meeting of ophthalmologists and pediatricians, and some interesting methods of attack on the disease developed from it.

The second symposium, which was sponsored jointly by the Sensory Diseases Study Section and the Microbiology and Immunology Study Section, was held last September in San Francisco to consider keratoconjunctivitis. So far as I know, this was the first time ophthalmologists and virologists had met together for such a purpose. The papers prepared for it and a summary of the discussions have been published in a volume that has evoked considerable interest and has been distributed widely throughout the world.

At this third meeting we have a union of ophthalmologists with physiologists, anatomists, biochemists, and others interested in visual mechanisms, and this should lead again to a great deal of interesting discussion and to a publication that will have wide distribution.

The committee that planned the meeting, headed by Dr. Crescitelli, should be congratulated on the excellence of the speakers they have brought together.

Chairman of the Sensory Diseases Study Section. University of California Medical School, The Medical Center (22).

Except for the minor changes necessary to adapt them to the typography of the ARCHIVES, *the papers in this symposium are published exactly as received, and neither the Editorial Board of the* ARCHIVES *nor the manuscript editing section of the American Medical Association can assume responsibility for their editorial style.*

The Lipoprotein Nature of Rhodopsin

NORMAN I. KRINSKY, Cambridge, Mass.

It seems appropriate to start a discussion of visual mechanisms with some comments on rhodopsin, the molecule which transduces light energy into the nervous impulse leading to vision. In particular, I shall discuss those aspects of rhodopsin which are concerned with its lipoprotein nature.

Lipoproteins as a class of complex molecules were first isolated in a pure form thirty years ago,[11] but we still know almost nothing about the mechanisms of association between the lipide and the protein portions of such molecules. The most common picture is of a molecule held together by electrostatic interaction between oppositely charged groups of the protein and phospholipides, which in turn, bind neutral lipides by secondary valence forces.

Despite this lack of precision in describing the mechanism of combination of lipides with proteins, a number of lipoproteins have been isolated with constant composition. The best known of these are present in human plasma, the so-called α- and β-lipoproteins.[15] These molecules contain 39 and 77 per cent lipides respectively, yet they exhibit the solubility properties and electrophoretic behavior of proteins. The only way one can visualize these molecules behaving as proteins is to assume that the peptide component is distributed over their surface, imparting protein properties to them.

Other compounds have been isolated containing roughly the same proportions of lipides as the plasma lipoproteins, yet exhibiting lipide properties, being soluble only in organic solvents and insoluble in aqueous systems. These are the so-called proteolipides which Folch and his associates[5] have isolated from the white and gray matter of the brain.

TABLE I.—*Lipoprotein Complexes*

	% Dry Weight		Reference
	Lipide	Protein	
Soluble Lipoproteins:			
Cenapse protein (horse serum)	41	59	12
α-lipoprotein (human plasma)	39	57	15
β-lipoprotein (human plasma)	77	23	16
Insoluble Lipoproteins:			
Thromboplastin (beef lung)	47		3
Chloroplast	30-44	50-60	18
Red blood cell ghost (beef)	36	64	17
Mitochondria (rat liver)	29	63	22
ETP	30		6
SDC	30-60		6
Cytochrome oxidase (beef heart)	33	67	13
Chlorophyll-lipoprotein	40		23
Retina (cattle)	16		4
Rod outer limbs (cattle)	28	38	4
Rod outer limbs (guinea pig)	40		20
Rod and cone outer limbs (monkey)	25		10

In addition to the soluble lipoproteins, there appears to be a large number of insoluble lipoproteins, usually found associated with highly organized structural components of various cells. Table I lists a number of these, together with the soluble lipoproteins.

In order to study the properties of these insoluble lipoproteins, it is necessary to use a solubilizer to bring them into solution. For this purpose, bile salts and natural and synthetic detergents have been used. In other cases, in particular the lipoproteins present in mitochondria, the particulate material is used as such, without attempting to bring it into true solution.

These insoluble lipoproteins contain from 25 to 60 per cent lipides. One example of this class is the enzyme, cytochrome oxidase, which can be isolated from heart tissue and contains 33 per cent lipide.[13]

Biological Laboratories, Harvard University.
Supported by funds from Harvard University.

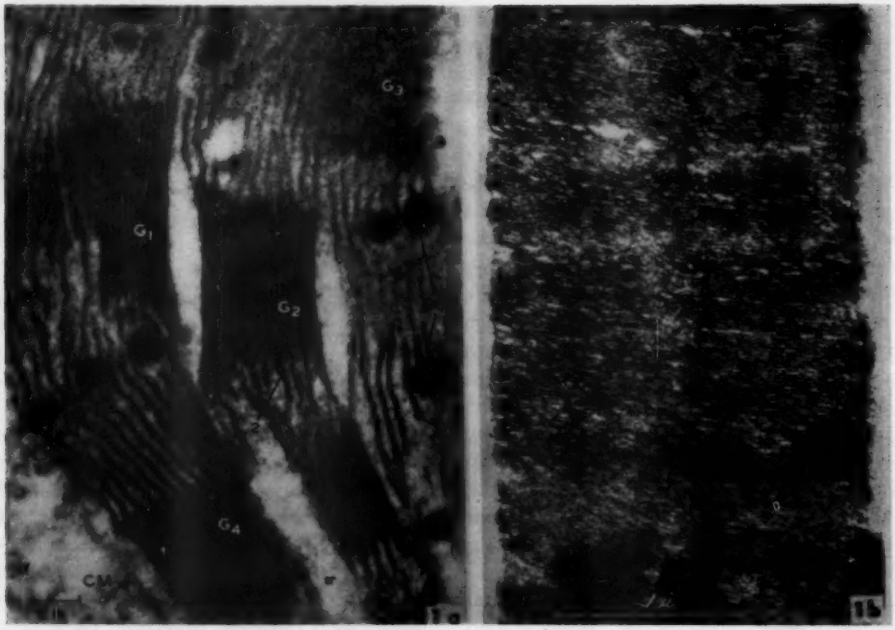


Figure 1.—Electron micrographs of a chloroplast and rod outer limb. (*a*) Cross-section through a mesophyll chloroplast from a 3-4 week-old maize leaf (7). (*b*) Cross-section through a perch rod outer limb (20).

This is an insoluble protein, and can be held in solution only with the help of bile salts or other detergents.

The mitochondria contain approximately 29 per cent lipide.[22] This cellular particle can be fractionated to give rise to certain sub-particles, such as the electron-transferring particle (ETP) and succinic dehydrogenase complex (SDC), which contain 20 to 60 per cent lipide.[6]

Figure 1 shows electron micrographs of two organized cellular structures which contain large amounts of protein-bound lipide. Figure 1a is a section through a mesophyll chloroplast from a 3-4 week old maize leaf, showing the lamellar structure of the grana and intergrana regions.[7] It is believed that these lamellae represent alternate layers of protein and lipide in a lipoprotein complex. Recently, a crystalline chlorophyll-lipoprotein has been isolated from chloroplasts, which is insoluble in aqueous systems, but can be brought into solution by means of various organic solvents.[23] This material contains 40 per cent lipide, approximately the proportion present in the whole chloroplast.

Figure 1b is an electron micrograph of a perch rod outer segment.[20] This also involves a lamellar structure of electron-dense bands, going completely across the rod outer limb. A very similar structure has been observed in cross-sections of nerve myelin sheaths, in which the lamellations are radially arranged.

The similarity between the rod outer limb and various other cellular structures can be extended to their chemical composition. Collins, Love and Morton[4] have shown that both frog and cattle rod outer limbs contain approximately 30 per cent phospholipides, a value similar to that found in many of the tissue lipoproteins which have been isolated. Like other tissue lipoproteins, rhodopsin, the visual pigment present in the rod outer limbs, is insoluble. It can be brought into aqueous solution with the aid of digitonin, and this is the method

of choice for studying its properties. The unit particle of such a solution is a rhodopsin-digitonin micelle, consisting of one molecule of rhodopsin and 180-200 molecules of digitonin.[8]

By differential centrifugation one can isolate rod outer limbs from cattle retinas, and extract the rhodopsin directly with a dilute aqueous solution of digitonin. When such solutions are extracted for total lipides,[21] we have found large amounts of phospholipides present. As the nitrogen to phosphorus ratio in these lipide extracts is approximately one, the phospholipides we are dealing with are primarily phosphatidyl choline (lecithin), phosphatidyl ethanolamine (cephalin) or phosphatidyl serine. Cholesterol, which is almost invariably present as a constituent of other lipoproteins, has not been detected in these extracts.

The presence of phospholipides in such extracts might however be due to impurities. One means of removing extraneous material from these solutions is through exhaustive dialysis, and this has been used with both rhodopsin and opsin preparations. Opsin is prepared like rhodopsin, except that bleached retinas are used as the starting material. Working with either rhodopsin or opsin, we found that approximately one-third of the phospholipides could be removed by dialysis, but the remaining two-thirds are bound tightly in the micelle.

Since large amounts of phospholipides were still associated with rhodopsin following dialysis, we tried extracting these solutions with organic solvents. This procedure usually involves precipitation of the protein followed by extraction of the lipides by the solvents. However, digitonin is such an efficient solubilizer of rhodopsin that it prevents the precipitation of the protein, making the subsequent extraction of lipides by organic solvents impossible.

To avoid this action of digitonin, we have used rod outer limb preparations directly. It has long been known that petroleum ether can extract lipides from lyophilized rod outer limbs without affecting rhodopsin. If the extraction is carried out below −10° C., polar organic solvents, such as acetone or diethyl ether can be used similarly. Table II shows the effect of two such solvents, and data on the lipide phosphorus and protein nitrogen content of these preparations. The effect of these solvents was twofold: they efficiently remove large quantities of phospholipides from the dried rod outer limb powders, and at the same time tend to render more protein soluble, so that the final digitonin solutions had less phospholipides, but frequently more protein than before this treatment. Thus, as Table II shows, acetone-diethyl ether at low temperatures removes two-thirds of the phospholipides associated with rhodopsin in the rod outer limb, but tends to increase the amount of protein solubilized by digitonin.

TABLE II.—*The Lipide Phosphorus and Protein Nitrogen Content of Various Rhodopsin Preparations*

Preparation	Lipide Phosphorus, Mole/Mole of Rhodopsin	Protein Nitrogen, Mole/Mole of Rhodopsin
Rod outer limbs	106	735
Digitonin extract of rod limbs	86	862
Digitonin extract of alum-hardened rod outer limbs	78	250
Digitonin extract of lyophilized and petroleum ether-extracted rod outer limbs	74	721
Digitonin extract of alum-hardened, lyophilized and petroleum ether-extracted rod outer limbs	54	205
Digitonin extract of acetone-diethyl ether powder of alum-hardened rod outer limbs	40	714

A number of other organic solvents, when tested on rod powders at low temperatures, irreversibly denature rhodopsin. Some of them—ethanol, phenol and butanol—are known to extract most of the lipides from a variety of tissues. Whether the effect of these solvents in denaturing rhodopsin is directly related to their ability to remove lipides is not yet known.

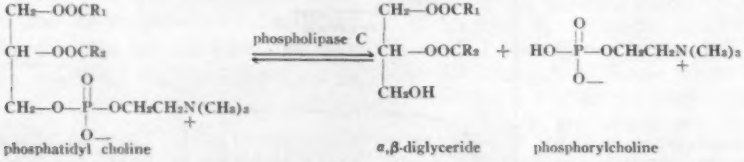
About fifteen years ago, it was observed that diethyl ether efficiently extracts lipides from tissues, when in contact with them at −20° C. or below, and allowed to warm to room temperature.[14] Under these condi-

tions, large amounts of lipides are removed from human plasma, without apparently denaturing any of the plasma proteins. For example, the isolated β-lipoprotein of human plasma yields all of its cholesterol and approximately half of its phospholipide to diethyl ether when treated in this way.[16]

We have used a similar procedure with rod outer limbs suspended in concentrated sucrose solutions. When these are shaken with diethyl ether and then frozen rapidly at $-70°$ C. we have found that approximately half the phospholipides are extracted on thawing. If this procedure is repeated a number of times in the dark, we soon reach a point at which very little further phospholipide is extracted. If the preparation is now exposed to light so as to bleach the rhodopsin, the next extraction yields more phospholipide than is extracted from a dark control.

The bleaching of rhodopsin in the rod outer limb therefore liberates phospholipides. It is not known how this change may be related to other changes in the properties of rhodopsin upon bleaching, observed previously: the appearance of sulfhydryl groups[26] and changes in the titration curve.[19]

Lipides associated with rhodopsin can also be removed by enzymatic hydrolysis. For this purpose, we chose phospholipase C present in extracts of *Clostridium perfringens*,[1] which catalyzes the following reaction:



Since the phosphorylcholine is water-soluble, the reaction can be readily followed by determining the rate of disappearance of lipide-soluble phosphorus.[9]

Digitonin was found to inhibit the activity of phospholipase C, when tested upon a preparation of purified egg lecithin. We therefore used rod outer limbs suspended in concentrated sucrose solution as the substrate for this enzyme. Figure 2 shows the course of the reaction of the *Cl. perfringens* extract on such a rod outer limb suspension. Following the addition of the enzyme, there is a rapid decrease in lipide-soluble phosphorus. The reaction does not proceed to completion, for a second addition of phospholipase C causes only a minor decrease in lipide-soluble phosphorus. The remaining phospholipide must therefore be either inaccessible to the enzyme, or lack the structural specificity that phospholipase C requires. The latter would be the case if the remaining phospholipides consisted of phosphatidyl ethanolamine or phosphatidyl serine.

Concomitant with the decrease in lipide-soluble phosphorus of the suspension of rod outer limbs, there was a rise in extinction. This increase in extinction, which occurs throughout the visible spectrum, is caused by an increase in turbidity, owing to the liberation of diglyceride from the phospholipide undergoing hydrolysis. The increase in turbidity can be used as a measure of enzymatic activity.

Under these conditions, we have been able to decrease the amount of phospholipide in the digitonin solutions prepared from phospholipase C-treated rod outer limbs to one-fifth their initial value, thus decreasing the molar ratio of lipide-soluble phosphorus to rhodopsin to approximately 20. This is the lowest ratio of lipide phosphorus to rhodopsin that we have yet obtained.

A molar ratio of 20 means that there are 14,000 gm. of phospholipide per mole of rhodopsin in the digitonin solution. As Table II shows, the lowest molar ratio of protein nitrogen to rhodopsin was 205.

[1] We are indebted to the Lederle Laboratories Division, American Cyanimid Company, for a gift of *Clostridium perfringens* Type A Filtrate.

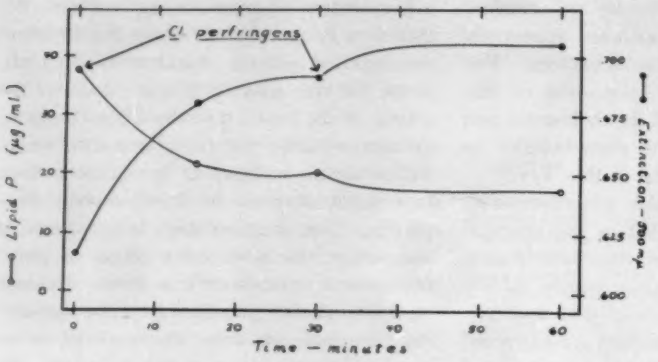


Figure 2.—The effect of a *Cl. perfringens* extract on the lipide phosphorous and extinction of a suspension of rod outer limbs (cattle) in 36% (w/v) sucrose.

Assuming that the protein contains 16 per cent nitrogen, this is equivalent to 18,000 gm. of protein per mole of rhodopsin. The molecular weight of rhodopsin would then be 32,000, of which 45 per cent would be phospholipide. This value is somewhat lower than Hubbard's estimate of the maximum molecular weight of rhodopsin.[8]

Although this enzyme does not bleach rhodopsin in the rod outer limbs, we found that digitonin extracts, prepared after the action of phospholipase C, were incapable of regenerating rhodopsin if the solutions were bleached and neo-*b* (11-*cis*) retinene was subsequently added to the solution.[27] Such spectral stability of rhodopsin, accompanied by an inability to regenerate the pigment, has been observed a number of times previously: for example, if rhodopsin solutions are treated with certain sulfhydryl poisons, such as *p*-chloromercuribenzoate[26]; and following acetylation of rhodopsin.[1] It appears, therefore, that hydrolysis of the majority of phospholipides in a rod outer limb suspension prevents subsequent regeneration of rhodopsin in digitonin solutions.

However, this is not the case in suspensions. Following the hydrolytic action of phospholipase C, the bleaching and regeneration of rhodopsin are carried out within the rod outer limbs. In Figure 3 we see the effect of phospholipase C on rod outer limbs, as followed by measuring the extinc-

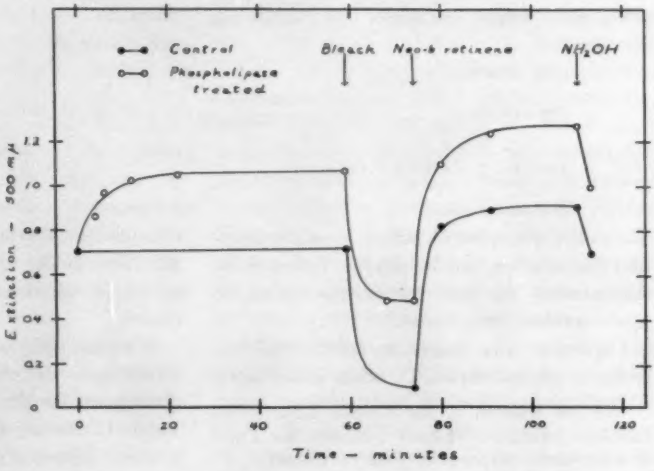


Figure 3.—The bleaching and regeneration of rhodopsin in phospholipase C treated rod outer limbs suspended in 36% (w/v) sucrose.

tion of the suspension at 500 mμ, the λ_{max} of rhodopsin. The rise in extinction of the control is due to the increase in turbidity mentioned earlier. After treating a suspension of rod outer limbs in sucrose for one hour with the *Cl. perfringens* extract, the preparation is bleached with orange (non-isomerizing) light to convert the rhodopsin to opsin and all-*trans* retinene. A control sample, containing no phospholipase C, is treated similarly. When all the rhodopsin had bleached, as shown by the decrease in extinction at 500 mμ, a small amount of neo-*b* retinene in acetone was added to the suspensions. There was a rapid increase in the extinction at 500 mμ in both the control and the phospholipase-treated sample, as rhodopsin regenerated. After 35 minutes in the presence of excess neo-*b* retinene, hydroxylamine was added to decompose any spurious compounds formed between retinene and the rod outer limb proteins. The amounts of rhodopsin re-synthesized were obtained from the differences in extinction between the hydroxylamine-treated and bleached samples. The control regenerated 100 per cent; the suspension treated with phospholipase C regenerated between 85 and 90 per cent. One can conclude that the removal of most of the phospholipides from rods has not altered the ability of the rhodopsin to regenerate.

The question still remains unanswered whether the remaining phospholipide is an essential component of the rhodopsin molecule. In this connection it is interesting to bear in mind that retinene extracted from rhodopsin solutions and brought into an organic solvent still, like bleached rhodopsin, is a pH-indicator.[24] This is apparently due to the formation of a Schiff base[2] between the aldehyde group of retinene and an amino group of an organic-soluble material, perhaps one of the phospholipides, phosphatidyl ethanolamine or phosphatidyl serine. This property is lost if retinene is purified by chromatography, separating it from other organic-soluble components.[25] Possibly in rhodopsin itself retinene is combined with the amino group of a lipide rather than of an amino acid.

We have seen that additional phospholipides are released from rod outer limbs following bleaching by light. This may be directly related to the initiation of the nervous impulse, for an alteration in the combination of phospholipides and proteins might markedly change the membranes of which the outer segments of rods are formed.

Biological Laboratories, Harvard University (38).

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DISCUSSION

CHAIRMAN CRESCITELLI: "Dr. Krinsky's paper is now open for discussion or comment. Would you please give your name and affiliation before making any remarks. Dr. Hagins, do you have any remarks you would like to make?"

DR. WILLIAM HAGINS, Naval Medical Res. Inst.: "Yes, I would like to ask Dr. Krinsky if this new value he has obtained for the molecular weight of rhodopsin is liable to affect the estimations for the number of retinene chromophores present in a single retinene-rhodopsin-digitonin complex."

DR. KRINSKY: "No, it would not, for two reasons. The first is that Hubbard assumed that a value of 40,000 was the upper limit for the molecular weight of rhodopsin and would be lowered if any protein impurities were removed in the preparation of rhodopsin. In the second place, she found that there was a one to one ratio of retinene to rhodopsin-digitonin micelles, indicating that there is only one retinene chromophore per molecule of rhodopsin."

DR. V. J. WULFF, Syracuse University: "I am a little confused about the relationship between lipid content and bleaching of rhodopsin. In some cases, removal of lipid causes bleaching; yet, in other cases, bleaching does not occur upon lipid removal. Are there specific types of lipids which are associated with this phenomenon?"

DR. KRINSKY: "When I said that removal of lipides bleached rhodopsin this was in reference to those organic solvents which irreversibly denature rhodopsin. It should be recalled that several of these solvents are capable of denaturing lipide-free proteins, so this effect could be either directly on the protein or through lipide removal. The phospholipase treatment, although it hydrolyzes a large amount of the phospholipide present in the rod, has no effect on either the spectrum or regenerability of rhodopsin. This treatment has lowered the lipide phosphorus/rhodopsin ratio to 20. This value may be critical, although further work may show that it can be decreased to zero. So far, all attempts to decrease it have resulted in denatured preparations. Even with the removal of large amounts of phospholipides, there has been no indication of an increase in the solubility of rhodopsin. This may be related to the fact that the phospholipase-treated rhodopsin still contains 45 per cent phospholipide.

DR. ZACHARIAS DISCHE, Columbia University: "I would like to ask Dr. Krinsky about one detail in his data on the relative ratio of phospholipids, nitrogen and rhodopsin. In your table presenting these data the protein nitrogen per mole rhodopsin in the water extract of alum hardened rod outer limbs was 259, but when the alum hardened limbs were extracted with acetone ether before extraction with water it went up to 715.

This means that you have some additional protein in the water extract after acetone ether extraction. I do not understand what that means because, apparently, by treating with alum, you remove a lot of inert protein which is not rhodopsin, is that right?"

DR. KRINSKY: "Alum treatment does not remove proteins; it merely 'hardens' or denatures them, preventing subsequent extraction."

DR. DISCHE: "But it becomes insoluble in water. If, however, you first treat this alum hardened material with acetone ether, you get that insolubilized material back in solution. Do you think that the acetone-ether treatment solubilizes again the proteins which were insolubilized by alum?"

DR. KRINSKY: "Not necessarily. It would seem more likely that a different protein, neither rhodopsin nor the type hardened by alum, had been sufficiently altered to permit its extraction by digitonin."

DR. DISCHE: "Isn't that a rather surprising thing, because digitonin acts as a detergent. The effect of the acetone-ether treatment would consist in the removal of lipids. Why should digitonin solubilize the protein denatured by alum after removal of lipids? This seems to me difficult to explain."

DR. KRINSKY: "Digitonin has a relatively selective action in solubilizing rhodopsin from rod outer limbs. If the protein moiety of a lipoprotein (insoluble before acetone-diethyl ether treatment) could now be extracted by digitonin, there would be an increase in the protein nitrogen of the extracts, even though there was a concomitant decrease in the lipide phosphorus."

DR. DISCHE: "Still, should it not also be the case if you treat the thing with pure salt solution without digitonin?"

DR. KRINSKY: "The use of salt solutions after the acetone-diethyl treatment has not resulted in the extraction of much additional protein material.

DR. DISCHE: "Are these the only proteins?"

DR. KRINSKY: "As long as we are dealing with a homogeneous micelle, we cannot tell if other proteins are present by the usual physical methods."

The Behavior of Flash-Illuminated Rhodopsin in Solution

VERNER J. WULFF, RALPH G. ADAMS, HENRY LINSCHITZ, and DONALD KENNEDY, Syracuse, N. Y.

Within the last 20 years, some aspects of the biochemistry of the visual pigments which bear directly on the visual process have become rather completely known. Especially significant have been the description of the bleaching process, the discovery of the chemical nature of retinene and its relationship to Vitamin A, and the elucidation of the conditions necessary for regeneration, including isomer specificity. As far as visual excitation is concerned, however, it is probably bleaching rather than resynthesis which is important. Studies on the chemistry of bleaching have always been handicapped by the slowness of techniques for recording absorption changes, and by the fact that prolonged bleaches have usually been necessary. Attempts to circumvent the former difficulty have been made by investigating bleaching with pigments in the dry state, or at extremely low temperatures; and though these experiments have yielded some interesting information, they involved conditions which are not physiological. In the present experiments, a recording system has been employed which allows one to follow absorption changes at room temperature only microseconds after photoexcitation[9] with an extremely short, intense flash. It is thus possible to study the behavior, at any desired time, of a population of molecules which have been excited nearly synchronously.[7] This paper describes some of the results obtained using this technique.

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Methods

The experiments were performed on cattle rhodopsin, extracted with digitonin from rod outer segments prepared from dark-adapted retinas.[11] Aliquots of the stock rhodopsin solution, buffered at pH 7, were introduced into a cylindrical absorption cell 5 cm. long and 1 cm. diameter and permitted to equilibrate to room temperature (27-28° C). Absorption spectra of the stock rhodopsin solutions were measured with a recording spectrophotometer.*

The filled absorption cell was placed in an optical system which permitted flash excitation[9] of the pigment and monitoring of changes in absorption during and after flash excitation (Fig. 1). An exciting flash of about 20μsec. duration and a computed energy of 65 joules was obtained by discharging condenser-stored electrical energy through 4 argon-filled flash tubes placed symmetrically around the absorption cell. A collimated light beam from a Zircon arc, attenuated by selected interference and neutral filters and controlled by a shutter, passed through the absorption cell, then through a monochromator and into a photomultiplier tube, the output of which served to produce vertical deflections of the cathode ray traces. The wave length band passed by the monochromator varied from 2 to 6 mμ and the deflection of the trace was linear with respect to transmitted light

*We wish to thank the Atomic Energy Commission, Division of Biology and Medicine, for the use of a Cary recording spectrophotometer.

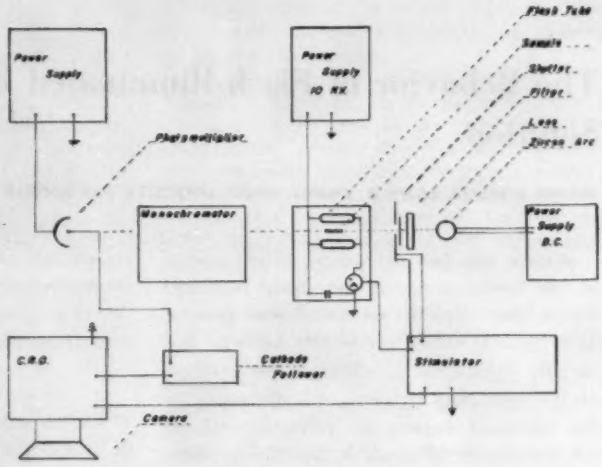

Fig. 1.—A schematic diagram illustrating the equipment and its arrangement.

intensity. Opening of the shutter in the analyzing light path served to initiate two pulses, separated by a suitable time interval; the first of these triggered the sweep of the cathode ray trace and the second initiated the exciting flash. The oscillographic response was photographed, providing a record of absorption changes at a series of wave lengths. Slower changes were followed by recording successive spectra in the Cary recording spectrophotometer. After each flash the absorption cell was emptied, rinsed, and refilled with stock rhodopsin solution.

Results

The nature of the data obtained is indicated by the oscillogram in Fig. 2. The

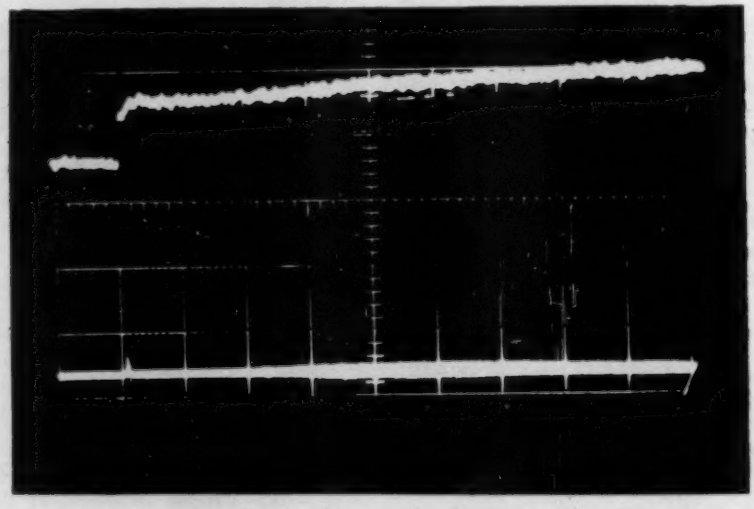

Fig. 2.—An oscillogram obtained at 516 mμ, with a sweep speed of 4 msec. per cm. on the tube face. The traces are read from left to right and the lower trace represents the zero-light transmission level while the upper trace measures the light transmitted through the rhodopsin solution before and after flash excitation. The sweep is triggered 4 msec. before the flash is produced (small spike on the lower trace indicates occurrence of the exciting flash and indicates the light scattered into the measuring pathway).

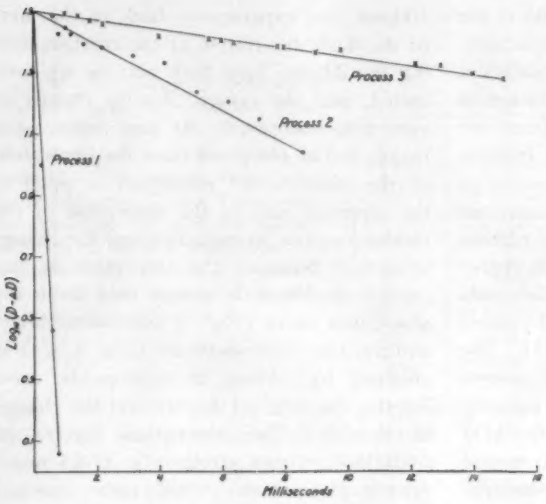

Fig. 3.—An analysis of the changes in light transmission following flash-excitation of rhodopsin as recorded in Fig. 2. The data, plotted as $\log_{10}$ (D—ΔD) vs. time (where D is the original density of the rhodopsin solution and ΔD is the change in optical density at any time after flash-excitation) indicate three distinct rates of change in the optical density of the solution. These three rates are identified as Process 1, 2 and 3. Process 4 is so slow that it does not appear at the sweep speed used. Zero time for process 3 is 15 msec. after onset of the flash, for process 2, 4 msec. and process 1 begins at zero-time.

upper trace measures the light transmitted through the absorption cell before and after the exciting flash, the occurrence of which is indicated by part of the discontinuity in the trace. The portion to the left of the discontinuity in the upper trace measures the light transmitted through the rhodopsin solution prior to photoexcitation; the rest of the trace measures the light transmitted through the solution for about 36 msec. following excitation. The lower trace is obtained without the analyzing light to determine the level of zero light transmission and to obtain a measure of the scattered light which reached the photomultiplier tube from the exciting flash alone.

The tracings in each oscillogram can be analyzed to yield the change in optical density of the photoexcited rhodopsin solution as a function of time. Since the distance between the zero-transmission line and the experimental tracing is a measure of the light transmitted through the rhodopsin solution, the ratio of light transmitted prior to excitation to the light transmitted at any time following photoexcitation can be com-

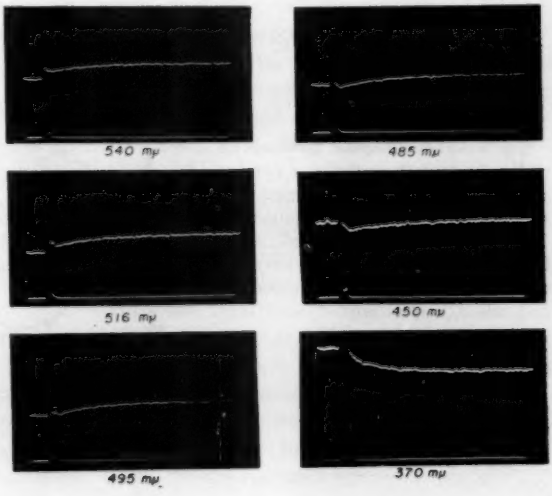

Fig. 4.—Six oscillograms obtained at 100μsec/cm. sweep speed illustrate the variation in the pattern of light transmission at different wave lengths following flash excitation.

puted. The logarithm$_{10}$ of this ratio is the change in optical density of the photoexcited rhodopsin solution. Fig. 3 shows a graph relating the log$_{10}$ of the change in optical density to time obtained from the oscillogram of Fig. 2. The data indicate three rates of change in optical density of the photoexcited rhodopsin solution: an initial rapid one which reaches a plateau 500μsec. after the onset of the flash (process 1), a second slower process which ends at 11 msec. (process 2) and a third process which ends at 100 msec. (process 3). The plateau between the first and second process can readily be seen in Fig. 2. The linearity of the relation between log$_{10}$ (D$-\Delta$D) and time suggests that the change in optical density is caused by a reduction in concentration of pigment molecules. The kinetics suggest that they are first-order reactions.

Absorption spectra of the rhodopsin solution at various times during and after photoexcitation may be reconstructed from a series of oscillograms such as those in Fig. 4. From such records, the change in optical density at any recorded time after the flash can be computed for the various wavelengths employed. By extrapolating the tracings for each wavelength in the 100μsec/cm. experiments back to the start of the flash, the course of the reaction during the 20μsec. light flash may be approximated, and the optical density change at zero time computed. At any given wave length and at any given time, the absorption of the photoexcited rhodopsin is equal to the algebraic sum of the absorption of the rhodopsin prior to excitation and the change in optical density. The absorption of unexcited rhodopsin is simply read from the absorption curve (Fig. 5, continuous line), and the zero time spectrum (Fig. 4, x's) is obtained by adding, at appropriate wave lengths, the original density and the change in density. The absorption spectra at t=500μsec. (open circles), at t=11 msec. (crosses) and at t=100 msec. (closed circles) are obtained in a similar manner.

The reconstructed absorption spectra indicate the changes which occur in the rhodopsin solution following flash excitation. Immediately upon photoexcitation, the absorption spectrum of the pigment changes; the photo-product shows λ_{max}=486 mμ and has an increased peak absorption (Fig. 5, x's). During the next 500μsec. there is a rapid decrease in absorption at 486 mμ, and a rise in absorption in the near ultraviolet.

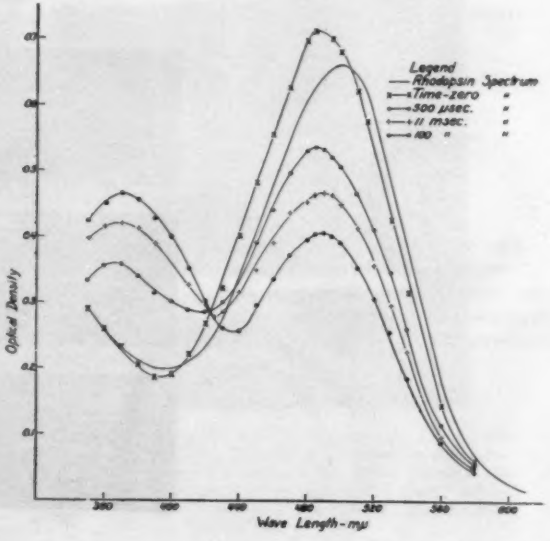

Fig. 5.—The absorption spectrum of rhodopsin prior to excitation (continuous line) and the reconstructed absorption spectra of the solution at 500μsec., 11 msec. and 100 msec. after flash excitation. These time intervals are at or near the end of processes 1, 2, and 3, respectively.

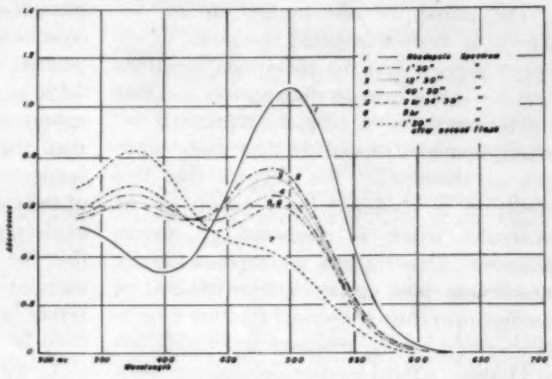

Fig. 6.—The absorption spectrum of rhodopsin prior to excitation (continuous line) and the recorded absorption spectra (broken lines) between 1.5′ and several hours after flash-illumination. Curve 7 represents the absorption 1.5′ after the solution measured in curve 6 was exposed to a second flash. Curves 2 through 5 indicate the slow changes which have been identified as process 4.

This is the first process indicated earlier in Figs. 2 & 3. During the ensuing 100 msec. two more, but slower, decreases in absorption take place, with concomitant rises in absorption in the ultraviolet. The reduction in the density at 486 mμ continues for some time in the dark (Fig. 6) accompanied by a slight shift in the λ_{max} toward the red, and finally yields a reaction mixture which is stable in the dark. This exhibits a λ_{max} at about 490 mμ (difference spectrum λ_{max}= 492 mμ), and has about ½ the peak absorption of the original rhodopsin solution. Exposure of this stable mixture to a second flash produces a further reduction in absorption in the visible spectrum and a corresponding increase in absorption in the near ultraviolet. Approximately the same sequence of slower changes takes place as those described above, including a shift of the absorption maximum of the flashed product towards longer wave lengths. The final product has λ_{max}=490 mμ and an extinction approximately ½ that of the solution before the flash.

Discussion

1. Transients in the bleaching of rhodopsin

Some of the changes described above resemble results obtained by others using different techniques. Lythgoe [8] and Broda & Goodeve [1] have described the spectral characteristics of an intermediate pigment obtained upon illumination of frog rhodopsin at dry ice temperatures. The λ_{max} of this orange-colored intermediate was displaced by about 5 mμ towards shorter λ's from that of rhodopsin; this absorption maximum disappeared when the solution was warmed. Wald et al.[10] performed a similar experiment with cattle rhodopsin, and described two intermediates: the first occurred at temperatures below −45°C upon illumination of rhodopsin, was characterized by an increase in extinction and a 5 mμ shift of λ_{max} toward the blue and was called lumi-rhodopsin; the second intermediate occurred upon warming the lumi-rhodopsin to −20°C and was characterized by an additional shift in λ_{max} of 7-9 mμ toward the blue. This substance was called meta-rhodopsin. Upon warming, the meta-rhodopsin disappeared, one half decomposing into opsin and retinene and the other half forming rhodopsin. Analogous results were obtained at room temperature upon illumination of a dry gelatin film impregnated with cattle rhodopsin. More recently Hagins [8] has employed the technique of flash-illumination of excised but intact eyes of albino rabbits. Changes in the absorption of retinal pigments were recorded oscillographically, using a beam of monochromatic light reflected out through the pupil of the eye. At 486 mμ, Hagins found a transient increase in absorption after the light flash followed by a reduction in absorption. At 400 mμ the absorption increases. These findings are in agreement with those presented here.

The results of our studies on the behavior of flash-illuminated rhodopsin in solution suggest that the absorption spectrum changes abruptly upon illumination and that the time-zero curve (Fig. 5) represents the absorption spectrum of the first photo-product of rhodopsin. We suggest that this spectrum is produced by a population of molecules which is composed of several fractions. One fraction decomposes rapidly to retinene plus opsin (within 100μsec. at room temperature), a second fraction decomposes more slowly, reaching an equilibrium at 11 msec., a third fraction decomposes more slowly yet, terminating about 100 msec., and a fourth fraction decomposes very slowly and terminates in about an hour. The undissociated pigment molecules which remain are light sensitive and exhibit an absorption maximum at about 490 mμ. This residual photosensitive pigment seems to be similar to that obtained by Wald et al.[10] and Collins and Morton [2] after illumination of rhodopsin at low temperatures.

2. Significance of the decay processes

An interesting question arises concerning the nature of the various fractions of the initial photo-product produced by the light flash. Perhaps the simplest explanation at present is that the light flash isomerizes the retinene moiety of rhodopsin as proposed by Hubbard and Kropf [6]; thus the four processes represent different isomers of retinene which dissociate from the protein at different rates.[5] Hubbard and Wald [4] indicate that the retinene which dissociates from rhodopsin after illumination with orange light is primarily all-trans retinene, as indicated by a fall in its extinction when it is subjected to iodine catalyzed light isomerization. Only when isomerizing light (light absorbed by the retinene) is used are other isomers of retinene obtained in appreciable quantities. The exciting light flash used in our experiments contained appreciable energy in the ultraviolet as well as in the visible spectrum and could, therefore, isomerize free retinene. If only all-trans retinene is released upon photo-excitation then the exciting flash may isomerize it sometime during the 20 microseconds of exposure. Presumably, this must occur while the retinene is still bound to the protein, since 50μsec. after the flash only 0.5% of the total free retinene which will appear is present. An alternative view is that the *protein-bound* neoretinene b is isomerized by white light yielding a mixture of the various isomers of retinene still bound to the protein. In either case, the hypothesis that the fractions present in the flash-illuminated rhodopsin solutions consist of different isomers of retinene bound to protein must be tested.

3. Probable nature of the residual photosensitive material

The residual photosensitive material obtained in our experiments is similar to that obtained by Collins and Morton [2] and Wald et al.[10], who warmed rhodopsin solutions after illumination at dry ice temperatures. The extinction of the photosensitive substance is about ½ that of the initial rhodopsin extinction, and λ_{max} is shifted toward the blue by about 5-6 mμ. Collins and Morton carried out successive low-temperature illuminations on the photosensitive material recovered from the initial bleach, and each time obtained a photosensitive residue exhibiting the same λ_{max} and final extinction about ½ that of the material illuminated. Hubbard and Wald [4] suggest that the photosensitive material recovered from low temperature bleaches is a mixture of rhodopsin and isorhodopsin.

Hubbard and Kropf [6] have recently presented evidence favoring the hypothesis that the first event in the bleaching process is an isomerization of the retinene chromophore. According to this view, a mixture of several isomers, bound to the protein at the chromophore site, would be formed. Of these, only two—the "neo-b" and "iso-a"—isomers will remain; the rest dissociate from the protein moiety. Thus there remains a residual photosensitive pigment which is probably a mixture of rhodopsin and isorhodopsin. This hypothesis appears to provide a reasonable explanation of the results presented here.

Thus, our present concept of the events which occur during and following photoexcitation of rhodopsin in solution is:

1. Flash-illumination results in isomerization of the chromophore of rhodopsin, producing a mixture of retinene isomers bound to protein.

2. Some of the isomers spontaneously dissociate from the protein component; the separate decay processes may suggest their separate release at different rates.

3. Two isomers, presumably neo-b and iso-a, remain coupled to the protein and constitute the residual photosensitive pigment; illumination of this residual pigment mixture results in a re-isomerization which is followed by the same sequence of changes.

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Differentiation, Metabolic Organization, and Viability of the Visual Cell

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Contents

Introduction
Methods
Visual Cell Differentiation to a Functional State
Respiratory and Glycolytic Changes During Postnatal Development
Recent Findings on Structure and Histochemistry of the Visual Cell
Visual Cell Death from Poisons
Phenomena Related to Iodoacetate Effect
Effects of Oxygen Poisoning and X-Radiation
Changes with Age in Cytocidal Effects
ERG Phenomena from Effects of Poisons
Degeneration of the Sensory Organelles
Hereditary Visual Cell Death in the C3H Mouse

In this paper I should like to review mainly studies of our laboratory on the physiology of the mamalian visual cell. These studies attempt to uncover the functional and metabolic organization of the visual cell as a unit and to determine consequences of this organization with regard to visual cell disease. Experimental approaches in this direction are most timely in view of the rapidly growing background of knowledge on structure and metabolism of cells in general and especially in view of the recent advances of our knowledge on fundamental properties of the visual cell. These advances concern the biochemistry of the photoexcitatory system, the microstructure of the visual cell as analyzed by electronmicroscopy and the histochemistry of the retina, to be reviewed briefly in the body of the paper. Nevertheless, one who wishes to study the visual cell in its natural environment is confronted with a multitude of difficulties in methods and interpretations. The function of the visual cell, for instance, must be ascertained by electrophysiological phenomena which still need much analysis as to their site of generation. This applies especially to a- and b-wave of the electroretinogram which in the studies to be reported were mainly used to detect changes in visual cell function. However, these manifestations of visual cell function can be measured so easily and rapidly that the visual cell actually provides advantages for analysis which are almost unique in cellular physiology.

Specific electroretinographic problems concerning localization and mechanism of origin of the component potentials will not be discussed in this paper. The ERG will

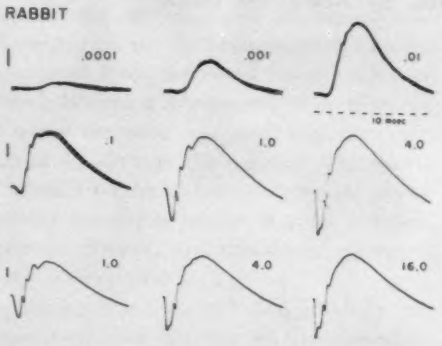

Fig. 1.—ERG during scotopic conditions in response to stimuli of different intensity. Routine test (see *METHODS*). The sweeps are triggered by the light stimulus, the start of the sweep coinciding with the stimulus. The numbers beside the tracings indicate the intensity of the stimulus. Intensity 1, 4 and 16 are according to the settings of the Grass stimulator. Numbers lower than 1.0 refer to stimuli at setting 1, their intensity reduced by neutral Wratten filter in the light path. The vertical line denotes .1 mV. and applies for the whole row. The length of each horizontal dash (see line under the upper right tracing) represents 10 milliseconds. All records are obtained with the same sweep speed. Five responses at each low intensity are photographed on single frame. Unless otherwise stated the following figures are arranged as fig. 1 and the experimental techniques used are the same.

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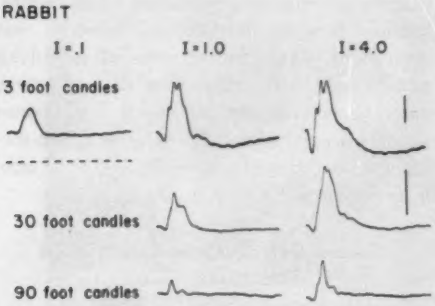


Fig. 2.—ERG during strong photopic conditions (rabbit). Typical b-wave activity has disappeared.

mainly serve as a tool to uncover changes in visual cell function. We will report on changes in function and metabolism during postnatal retinal development,[1,2] on the effects of metabolic poisons[3-11] and on the ERG in experimental and hereditary (mouse) visual cell degeneration. The goal of this review is to provide background information on properties of the visual cell which might be related to causes and phenomena of hereditary visual cell diseases in man. Since the photochemical system has been most authoritatively discussed recently in relation to this disease it will not be included in our consideration.[12,13]

Methods

Electroretinography.—The multitude of phenomena which the electroretinogram (ERG) records in response to illumination makes it necessary to select certain suitable ones for a routine testing of visual cell function. In the studies to be reported, only that fraction of the ERG which occurs within 200 msec. after the "on" of illumination was generally measured. Figures 1 to 3 show the main portions of this test for the curarized and artificially ventilated rabbit. It includes the recording of responses provoked in the fully dark adapted animal by light intensities ranging from threshold to the maximum available. The test further includes recordings during the light-adapted state, and it ends with flicker stimulation at a high stimulus intensity. If conditions do not permit to perform the whole test e. g. during the rapid action of a poison the various phases of the test are spread over different experiments. The light source of this test is the Grass Photic stimulator at a reflector distance of 12". The duration of the flash is about 10 microseconds; the maximal peak intensity (according to the manufacturer) more than 10^6 candle power. The total time constant of amplifier and cathode ray oscilloscope is set at more than 500 msec. The amplification used is the same in each experiment but differs for different parts of the test. The cathode ray sweep speed for all responses is 20 msec/cm. but selected responses are recorded in addition at 10 msec/cm.

The average shortest latency of the a-wave (at maximal stimulus intensity) of the curarized, adult rabbit was in this test 2 msec.; the longest measurable (sub-maximal stimulus) approximately 40 msec. The b-wave latency in the fully dark adapted animal ranged from 12 msec. (max. stim.) to 45 msec. (at .5 log units above threshold intensity). Maximal b-waves amplitudes were around .6mV., those of the a-wave .25mV.

In the routine experiments the ERG was obtained by cotton electrode in contact with the anesthetized cornea. The pupil was maximally dilated by atropine and the eye

Fig. 3.—ERG during flicker (rabbit). Each picture of the top row shows the first 5 responses to 1/sec. flicker at the indicated intensities. The b-wave in response to the 2nd and following stimuli is curtailed, the degree of this curtailment depending upon stimulus intensity. Under abnormal condition (chronic iodate effect) this curtailment may fail to appear. Bottom row shows normal responses to different rates of flicker at intensity 4. The fast potentials recorded are a characteristic feature of the rabbit's ERG; fusion occurs at a frequency higher than 100/sec.

kept wide open. A short but also standardized testing procedure was employed if the same rabbit was to be repeatedly tested over several days or weeks. No curare or general anesthesia was used in these tests. In very young rabbits adequate fixation of the head was impossible; they were for repeated testing examined under flaxedil relaxation and during maintenance of their respiration by a body respirator. Mice were tested under Nembutal anesthesia. The rabbits used were exclusively (albino) New Zealand Whites.

In vitro measurements.—Rabbit retinas at different ages were studied, under various conditions, mainly for respiratory and glycolytic activity and ability to oxidize glucose to carbon dioxide. The media used were Krebs-Ringer-phosphate buffer [14] and that described by Ames and Hastings,[15] which is a bicarbonate-buffered medium resembling human cerebrospinal fluid in composition. Respiration was measured by conventional manometric methods. Glycolysis was measured not only manometrically, but by the determination of lactic acid with the enzymatic method of Horn and Bruns,[16] modified to permit routine analysis of microquantities. Glucose oxidation was measured by the method Wenner and Weinhouse [17] using C^{14}-labelled glucose. The metabolic CO_2 was precipitated as $BaCO_3$ and counted at infinite thickness.

For removal of the retina the eye was placed in a special cup of perforated stainless steel where it could be held in place by gentle suction. The front portion of the eye was sliced off just posterior to the *ora serrata.* The posterior hemisphere was everted on a suitably-shaped rubber stopper affixed to a rod and pinned down at three points of the periphery. The everted eye cup was submerged in the experimental medium and revolved while the retina was teased free with a smooth glass spatula until it was hanging by the optic nerve. Then it was cut free into the measuring flask. The flask was immediately affixed to a manometer and placed in the bath to equilibrate. In some experiments the Warburg flask and medium were kept warm until the retina was added, thereby permitting a reduction of the equilibration time to 5 minutes. When it was desired to obtain initial rates of glycolysis immediately after dissection of the retina, manometry was not employed but instead samples were taken from the incubation medium at 10 minute intervals for lactic acid determination.

Visual Cell Differentiation to a Functional State

The morphological differentiation of the visual cells occurs in many mammals during the early postnatal period so that an excellent opportunity presents itself to study the cell while it undergoes the changes from a primitive form to its complex adult appearance.

Morphology.—Figure 4 informs on the changes in retinal histology between the age of 2 days to 6 weeks as they occur in the albino rabbit. They generally follow the same pattern as described for rat,[18] mouse [19] and dog.[20] Species differences exist as to the state of the retina at birth and the exact time course of development.

At the age of 2 days, the separation of the rabbit's retina into three cellular layers has just been achieved. The ganglion cells at this age are already of adult appearance but a great fraction of the bipolar cells and almost all outer nuclei are still immature as evidenced by the ovoid form of the nuclei and the granular distribution of their chromatin. At both sides of the outer limiting membrane small rod shaped protoplasmatic elements—buds of the sensory organelles—have made their first appearance.

During the next days, the process of retinal differentiation still occurs mainly vitread the outer limiting membrane. At 7 days the inner nuclei have much matured, the outer plexiform layer has become more distinct and many visual cell nuclei in the inner half of the layer show clumping of their chromatin similar to that which characterizes the adult visual cell nucleus in Zenker fixed material. The budding sensory organelles have increased in number but only

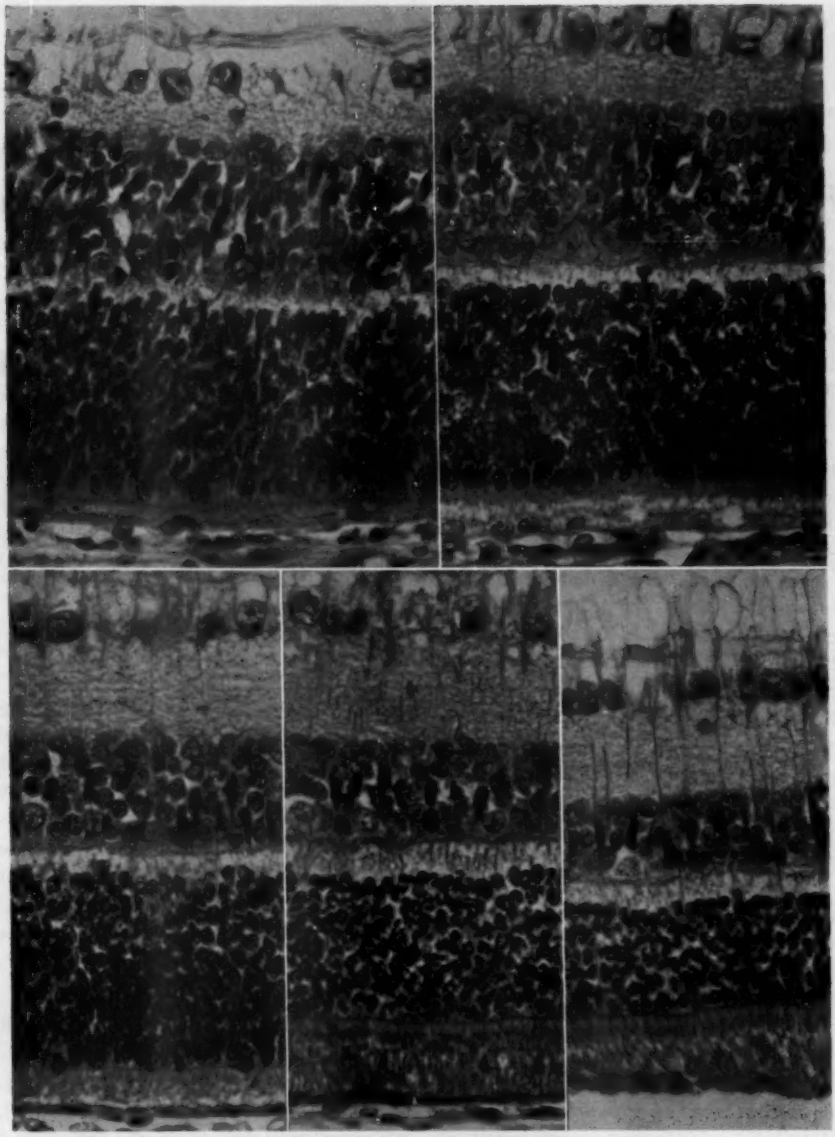


Fig. 4.—Rabbit's retina at different ages. Top row from left to right, retina at 2 days and at 7 days. Bottom row retinas from ages of 11 days, 20 days and 42 days. The sections are from the central region. Hematoxylin-eosin. Objective × 50 (oil).

slightly in length. However, in the most advanced retinal region one can, at this time, distinguish by ordinary histology between a proximal part of the sensory organelle which carries a small ellipsoid body and a distal part which is faintly stained and thinner than the ellipsoid region. At a slightly more advanced stage, generally at 8-9 days after birth, a miniature sensory organelle has clearly developed having an outer limb of about the same length as the proximal segment of the organelle. The total length of

the miniature organelle is then about 15% that of the adult organelle.

As soon as the development of miniature organelles has become uniform over a retinal region, the growth of the organelles accelerates. It is particularly fast during the age of 11 to 18 days during which period the retina becomes more and more adult-like in its appearance. These changes occur later in the periphery of the rabbit's retina than in the central region to which the time relationships given above apply. The bulk of the peripheral visual cells follows in development the central region by 1 to 2 days whereas the cells at the ora serrata are 3 to 5 days behind the central ones. Development through the ventral part of the retina, moreover, is faster than in the retina dorsal to the optic nerve. However, at about 25 days practically all visual cells have reached the same stage of development and differences which exist in the length of the organelle are proportionate to those which the adult retina shows. The length of the sensory organelle is then about 70% that of the adult.

The further growth of the organelles and other retinal changes proceed slowly over several weeks and are closely associated with the increase in eye size. At 20 days, the eye diameter is about ⅔ that of the adult. It takes more than 2 months until it is within 5 to 10% that of the full grown animals. Simultaneously the retinal layers vitread the outer limiting membrane become thinner while the sensory organelles thicken apparently to fill out the expanding retinal surface. In conjunction with this thickening, their length increases.

The earliest signs of retinal functioning.—The ERG makes its first appearance at about the 8th day when miniature sensory organelles have developed in the central region. Apparently as soon as ordinary histology can clearly differentiate between outer and inner limb, the sensory organelle is ready to assume function. As illustrated in Figure 5 the early ERG consists mainly of a cornea-negative potential which is very similar in

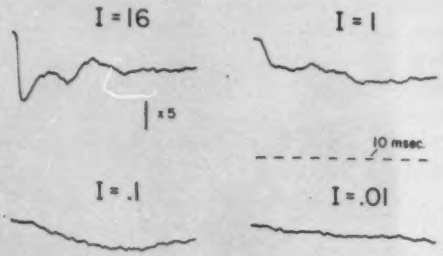


Fig. 5.—Rabbit's ERG at age of 9 days. Note the increase in a-wave latency with the decrease in stimulus intensity; a b-wave is not yet evident.

shape to the isolated a-wave (after b-wave elimination) of the adult retina. Its initial slope is slower but nevertheless the potential develops surprisingly fast. At the age of 9 days its maximal amplitude is about .05 mV. and responses can be provoked for about 3 log units from maximal. The latency of this early a-wave at maximal stimulus intensity also is relatively short and is no more than twice that of the adult wave.

A b-wave cannot be detected at this early stage. Instead two distinct waves of a duration of about 50 msec. follow the swing to cornea-negativity (Fig. 5). They are evident only when intense stimulation is used. With further progress of retinal development these waves become faster and after the b-wave has become predominant they merge with the rising phase of this potential. Since these waves are so prominent in the ERG of the immature retina they should represent an essential component of the retinal excitatory processes.

The whole pathway from visual cell to ganglion cell functions when the ERG has made its first appearance. In the experiments of Figure 6 the free tip of a 10μ platinum electrode is at the inner surface of the central region of the retina of a 9 day old rabbit under flaxedil after removal of the cornea and part of the lens. Ganglion cell discharges occur in response to illumination even at a light intensity which is at least 1 log unit subliminal for ERG production. Spontaneous ganglion cell activity also has developed at this time. Furthermore,

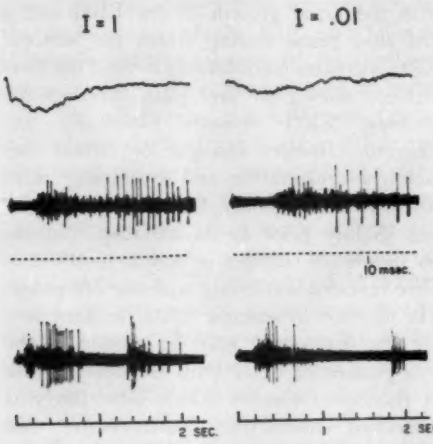


Fig. 6.—Ganglion cell discharges in response to the indicated light intensities at a very early stage of retinal functioning (rabbit at age of 9 days). Activity appears predominantly in form of bursts of discharges; excitation apparently alternates with inhibition. The last burst of activity can occur as late as 5-10 seconds after the brief flash. Note that the different elements participate to a varying degree in burst activity. Upper row and middle row are recorded with the same sweep speed. The ERGs were obtained prior to the insertion of the microelectrode. Ganglion cell responses in this experiment were evident at I=.0007; no ERG was obtained at this low intensity.

excitatory and inhibitory phenomena in response to illumination are evident.

Similar records as in Figure 6 were obtained at an age of 8 days but no ganglion cell activity was detected at 6 days or even at 7 days. The neural pathways therefore either attain a functional state simultaneously with the development of a miniature sensory organelle or waited for outer and inner limb to become functional. Gross histology seems to suggest that bipolar cells and ganglion cells are ready to function prior to the emergence of a miniature sensory organelle, but no such suggestion can be made with respect to the synaptic ending of the visual cell which needs electronmicroscopical technique for its study. Nevertheless, the development of the sensory organelle to a functional state must be very closely timed with the development of all other parts of the cell which serve excitation.

The growth of the ERG.—ERG development has essentially two aspects 1) the general increase in amplitudes and reaction rates and 2) its maturation to the adult relationship between a- and b-wave. The most rapid phase of ERG development [1] in both respects occurs between 11 and 18 days coinciding with that period of histological development during which the sensory organelles grow from about 25% to 70% their adult length and during which the outer nuclear layer attains adult appearance. At 11 days (Fig. 7) a b-wave has clearly developed but the a-wave still is very prominent mainly as the result of the slow slope and of the relatively late emergence of the b-wave. At 18 days the ERG resembles that of the adult but is still markedly deficient in b-wave when tested by low stimuli during scotopic conditions (see top rows of Fig. 7). These differences slowly disappear. Roughly coinciding with the thickening and further increase in length of the sensory organelle, b-wave amplitude

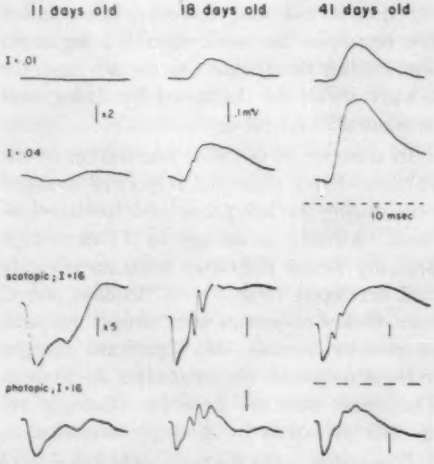


Fig. 7.—Electroretinogram at three stages of development. Each column represents one experiment; the animals were under Flaxedil and artificially ventilated (trachea-cannula). The upper 3 rows are recorded during scotopic condition. Intensity of the light flash is the same in each row. Amplification of the ERGs of the 11 day old rabbit is higher than that of the ERGs at the other ages. It was twice increased for the records of the top rows, and five times for those of the bottom rows.

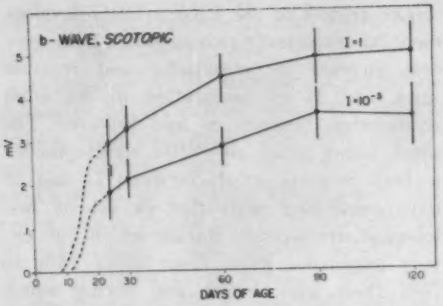


Fig. 8.—The b-wave amplitude related to age at two different stimulus intensities. The stimulus in the experiments was a 120 msec. tungsten light flash. The animals were fully dark-adapted; no general anesthesia, no muscle relaxant. Each point represents the average of 8-10 experiments; the vertical lines denote total spread of the measurements. The dotted lines to the left indicate the probable growth of the ERG prior to 25 days according to measurements obtained with a different technique (Flaxedil, artificial respiration).

increases steadily up to an age of 80-90 days. In measurements performed with the same technique and under the same conditions the maximal b-wave amplitude almost doubled during the age of 25 to 90 days (Fig. 8). The continuity in b-wave rise during this period was, however, best evident for responses no more than 3½ log units above adult threshold. On the average, the b-wave threshold decreased by 1 log unit between 25 and 90 days.

In contrast to the slow maturation of the scotopic ERG photopic responses changed little during the late phase of ERG development. Already at an age of 11 days high intensity flicker responses were surprisingly well developed (Fig. 9). At 18 days, moreover, flicker responses were almost the same as in older animals. No significant changes in these responses occurred after 30-90 days. The same was the case for photopic responses provoked by a single stimulus.

Discussion.—On the basis of histology and ERG measurements postnatal visual cell differentiation can be divided into three phases: first, a phase during which the cell develops a miniature sensory organelle and develops a minimal function; 2nd, a phase during which the sensory organelle rapidly approaches adult appearance simultaneously with the rapid growth of the ERG and a 3rd slow phase during which the scotopic ERG increases considerably beyond the level attained during the 2nd phase whereas the photopic ERG remains practically unchanged. *In-vitro* changes of retinal metabolism (respiration and glycolysis) were found to occur mainly during the 2nd phase and slightly prior to its initiation. So far no significant changes in retinal metabolism were revealed coinciding with the 3rd phase. The increase in scotopic ERG function during the 3rd phase may be related to the enlargement of outer limb and the increase in rhodopsin content which most likely is associated therewith. Furthermore, this phase was found to coincide with the development of the specific susceptibility of the visual cell to iodoacetate and oxygen poisoning. General cellular changes, therefore, seem to accompany the development of maximal scotopic efficiency.

Due to differences in technique our measurements place the first appearance of the ERG at an earlier time of visual cell development than those of the literature.[21,22,23,24] For probably the same reason the late maturation of scotopic responsiveness was more pronounced in our study. Our findings differ, however, beyond such simple ex-

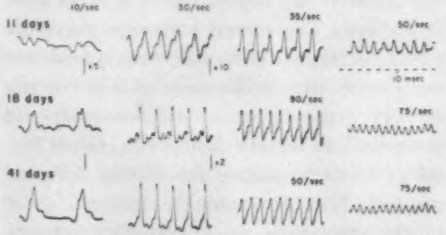


Fig. 9.—Flicker responses at different ages. Top row—responses at the age of 11 days; middle row—18 days; bottom row—41 days. Continuation of the experiments illustrated in figure 7. Stimulus intensity is I=4 (see legend of fig. 1). At the age of 11 days, 50/sec. flicker produces almost the same type of response as 75/sec. at 18 and 41 days. Differences in responsiveness between 18 days and 41 days are minimal. Numbers beside calibration marks indicate by what factor the length of the line should be increased in order to represent a signal of .1 mV. Amplification at 11 days is 5 times higher than for the corresponding records at 18 and 41 days.

planation from those of Zetterström [25-27] on the development of the ERG in children. Although most visual cells of the newborn infant are well differentiated and undoubtedly are functioning, the ERG was practically absent during the first days of life and very small during the following weeks. The deficiencies involved the a-wave even more than the b-wave. They were evident for photopic as well as scotopic responses. It thus seems as if ERG development in man is mostly one which corresponds to the third phase of development in the rabbit, the earlier phases being completely absent. Compared with the rabbit, man should have an ERG at birth of about one half the amplitude one records in the adult provided the techniques are comparable. Further studies on this problem are definitely indicated because of their importance for clinical electroretinography and because they may suggest as Zetterström [28] points out that the development of visual cell metabolism in man is hindered by factors which do not become evident in lower animals.

Respiratory and Glycolytic Changes During Postnatal Development

It is to be expected that the development of the retina to a functional state is associated with numerous biochemical changes. The specific energetic requirements of the functional state are to be satisfied and in conjunction with structural differentiation new chemical systems may be developed which are specifically concerned with visual and nervous excitation. In contrast to a wealth of information on the biochemical development of the brain (see Ref. 29) studies of the retina during differentiation are practically missing. In the chicken Krebs [30] and Tamiya [31] measured glycolysis at various early ages, while Rudnick and Waelsch [32] presented data on the development of an enzyme of glutamine synthesis. Both measurements revealed changes of amazing magnitude during the maturation of the retina.

To us, the study of postnatal retinal metabolism in a mammal was of particular interest because of the unusual susceptibilities of the visual cells of the adult retina to certain poisons and because of the possibility that hereditary visual cell degeneration might be related to a specific defect in energy yielding reactions. Since the gross morphological differentiation of the visual cells is late in relation to the differentiation of the inner layers it was hoped that the study of the metabolic changes of the retina might give information on specific metabolic properties of the visual cell. To this end, Dr. Cohen measured *in-vitro* respiration, glycolysis and glucose oxidation of the rabbit's retina at ages between 2 days and several months. From these studies there has emerged the following picture.

The young retina consumes less oxygen and also produces less lactic acid than the adult retina. Respiration increases most rapidly between 12 and 20 days after birth. This is approximately the same period of time during which outer and inner limbs grow most rapidly and during which the ERG undergoes its fastest change (2nd phase of ERG development). The magnitude of the respiratory increase depends upon the medium used and other experimental conditions. In Krebs-Ringer-phosphate-glucose the respiration of the adult retina related to dry weight is about 3 times higher than at 2-5 days or about 5 times higher per whole retina. About 70% of this change occurs between 12 and 20 days.

The adult retina produces under aerobic conditions about twice as much lactic acid per mg. dry weight than the very young one. Anaerobic glycolysis is about 1.6 times higher in the adult. This increase in lactic acid production occurs earlier than that of respiration mainly between 7 and 12 days. This period coincides with that during which the miniature sensory organelles appear all over the retina and a primitive ERG develops.

The changes during the postnatal development of the retina in respiration and glycolysis are not unlike those measured for

	Respiration			Glycolysis		Glucose Oxidation	
	Q_{O_2} in 0.02 M Glucose	Stimulation by DNP in 0.02 M Glucose	Crabtree Effect	Q^{air}_{LA}	$\frac{Q^{N_2}_{LA}}{Q^{air}_{LA}}$	μatoms C per mg. d. w.	Proportion of O_2 Consumed
7 day	3.5	2.3x	32%	22	1.5	0.054	35%
Adult	9.2	2.5x	10%	43	1.3	0.28	67%
IAA-retina	8.1	----	25%	32	1.9	0.20	55%

Q values are expressed as μl/hr/mg. dry weight but were measured during a 10 minute incubation period, and approach initial rates. Medium: Krebs-Ringer-phosphate with 0.02 M glucose. The Crabtree effect is expressed as per cent inhibition of respiration by glucose.

the brain or to be assumed for the brain from enzyme determinations. For instance, succinic dehydrogenase, cytochrome oxidase and ATPase for the whole brain of rats are at a low level of activity until about 6 days after birth; they increase suddenly 3-6 times between 7 and 15 days and have reached at the end of this time about 80% of the adult levels.[33] Aerobic lactic acid production of rat's cerebral hemisphere, measured in the same medium as the retinas to which the above values apply, increases sharply about 3 times (per fresh weight) between 10 and 20 days and then remains practically constant.[34] These two examples may suffice to indicate that the retinal changes are not of outstanding magnitude and occur at about the same time as the main cerebral changes (illustrating the high degree of coordination of nervous development). The pattern of retinal development appears to be more precise than that of the brain as one would expect from the more synchronized course of retinal development.

The retina is distinguished throughout the postnatal period by the preponderance of lactic acid production over oxygen consumption. This exceptional property of the retina (see Table) is as evident at birth as it is in the adult. As is well known the retina resembles cancer tissue very strikingly in its high glycolysis.

It is generally assumed that the main postnatal cerebral change in respiration is related to the simultaneous growth of the dendrites and the synaptic networks. By the same token visual cell differentiation may be largely responsible for the observed increase in retinal respiration and glycolysis. For instance, the increase in the size of the ellipsoid body, where a high respiratory capacity seems to reside, may account for a great fraction of the respiratory change between 12 and 20 days. Similarly, the earlier increase in glycolysis might be related to this development of the proximal fibers and synaptic terminals of the visual cells (where glycolysis is probably the main source of energy) in readiness to function as soon as the sensory organelles have differentiated. These are speculations, however, and in order to ascertain where the main changes in metabolism occur, it is necessary to know to what extent visual cell metabolism differs from that of the inner layers.

To this end, retinas which had no visual cells as a result of poisoning by iodoacetate were used. Iodoacetate was intravenously administered to adult rabbits in doses just sufficient to damage irreversibly the visual cells. Within a few days, the visual cells underwent lysis and disappeared from the retina leaving no trace while the bipolar cells (inner nuclear layer) and the ganglion cells remained intact. Two to three weeks after the administration of the poison, the retina which now lacked the visual cells was removed and measured by the same technique as other retinas. The inner layers of a rabbit's retina comprise about one half the volume of the whole retina. Using this value and the measured activities of IAA

and normal retinas one can by simple arithmetic arrive at an estimate of the *in-vitro* activity of the outer retinal layers.

In experiments several years ago with N. Powell it was found that the IAA retinas respired slightly more in bicarbonate buffer than normal retinas whereas their aerobic lactic acid production was considerably lower when compared on the basis of dry weight.[35] In the present series of experiments respiration was slightly lower than in the normal adult; aerobic glycolysis was reduced by 25% but anaerobic glycolysis was the same (Table). We therefore conclude that at the time of visual cell differentiation all layers of the retina undergo an increase in respiration, and also in anaerobic glycolytic capacity.

Some important differences in organization of metabolic activities between young and adult retina were found during these studies. As shown in the Table, the oxidation of the glucose of the medium to CO_2 accounted in the 7 day retina for only 35% of the oxygen consumed as measured with uniformly C^{14}-labeled glucose. Glucose oxidation accounted for 55% of the oxygen consumed in the IAA retina and for about 70% in the normal adult retina. Clearly, the main substrate for respiration in the young retina is stored material. It is very probable that these stores are non-carbohydrate but that they are nevertheless replenished in part from glucose molecules which are neither directly oxidized to carbon dioxide and water nor converted to lactic acid. On the other hand, direct oxidation of the added glucose predominates after postnatal development but even then is not the sole source of respiration.

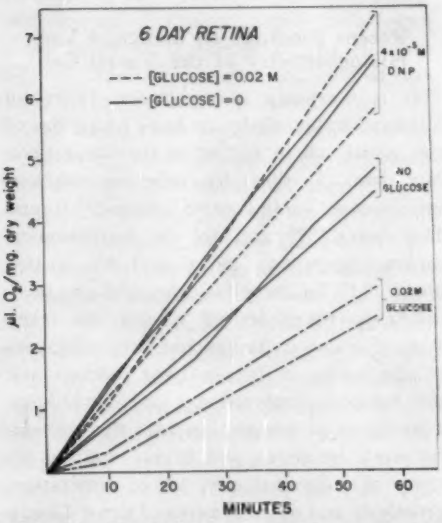


Fig. 10.—The Crabtree effect in the young retina. Each flasks (5 ml. capacity) contained one retina in 0.7 ml. of calcium-free Krebs-Ringer-Phosphate at pH 7.4. The uppermost set of curves show that in the presence of 2,4-dinitrophenol, respiration is unaffected by glucose.

A second difference between young and adult retinas concerns the effect of added glucose upon the amount of oxygen consumed. Cohen [2] found as illustrated in Figure 10 that the young retina respires less when the medium contains physiological concentration of glucose than when it lacks glucose or any other substrate. This inhibition by glucose amounted to 32% in the 7 day old retina. However, it was only 10% in the adult retina but consistently 25% in the IAA retina (see Table). A similar effect of glucose had been known hitherto only for cancer tissue and was believed to be a special property of cancer metabolism. It was first reported by Crabtree [36] and has recently been studied extensively with tumor ascites cells.[37-42] Since this "Crabtree effect" and its disappearance during postnatal development relates to the metabolic organization of the visual cell it will be discussed in greater detail.

According to Brin and McKee [37] and Racker and his co-workers [38,39,40] the Crabtree inhibition by glucose is principally due to competition between respiration and a high-capacity glycolytic system for phosphorylative cofactors such as adenosine diphosphate (ADP) and inorganic phosphate. Glucose, by putting the glycolytic capacity to work, seems to decrease the level of ADP or inorganic phosphate or both thereby imposing a limitation upon the respiratory chain.

The existence of such a competition between glycolysis and respiration through

phosphorylative cofactors is supported by the fact that uncoupling agents abolish the inhibition of respiration by glucose, a finding which was also observed with the young retina (Fig. 10). The principal evidence that inorganic phosphate is involved is twofold: the addition of glucose to a suspension of ascites cells results in a depression of intracellular inorganic phosphate[39] and secondly, the inhibition of respiration by glucose is partly relieved by high levels of inorganic phosphate in the medium.[37,38] Evidence pointing to ADP as the focal point of glucose inhibition was reported by Chance and Hess.[41] Of interest in this connection are the experiments of Gatt et al.[40] with a reconstructed cell free system from purified mitochondria and crystalline glycolytic enzymes with their cofactors. In such a system respiration was inhibited by glucose but the omission of a single glycolytic enzyme abolished this inhibition, showing that in this system glucose exerts its effect indirectly, through the glycolytic mechanism. There are two phenomena which seem to be in disagreement with the "competition" theory. Firstly, glucose inhibits respiration even when lactic acid production is blocked by iodoacetate[38,39]; secondly, the glucose concentrations required for near-maximal lactic acid formation are lower than those required to produce a pronounced Crabtree effect.[42] However, both observations can be reconciled with the competition theory, if one assumes that the initial steps of the glycolytic system are involved in the Crabtree effect.

On the basis of this theory we assume that in the young retina competition exists between respiration and very active glycolytic enzymes. The properties of the young retina which enable the manifestation of a Crabtree effect seem to be retained after postnatal development by the inner layers as suggested by the manifestation of this effect in the IAA retina. But the small extent to which this effect is apparent in the normal adult retina despite its high glycolytic activity indicates that the adult visual cells lack this competition between respiration and glycolysis or a portion thereof. The effect measured with the adult retina can be accounted for completely by the magnitude of the effect in the isolated inner layers. In a similar way, the small difference between aerobic and anaerobic glycolysis of the adult suggests that glycolysis and respiration are not intimately related in adult visual cells (see Table). It is important to note that the stimulating effect of 2,4-dinitrophenol on respiration was just as high in the adult as at 7 days (Table). It would thus appear that our experimental conditions did not selectively impair coupling of respiration and phosphorylation in the adult.

We conclude that postnatal differentiation leads to a metabolic organization of the visual cell where a respiratory system resides in the same cell together with a high capacity glycolytic system in apparent functional separation. Physiological data on visual cell function and viability during impaired metabolism *in-vivo* point to the same type of organization and selective effects of poisons upon the adult visual cells may have as their basis this particular and exceptional organization.

Recent Findings on Structure and Histochemistry of the Visual Cell

It is necessary to review briefly recent advances in knowledge on basic properties of the visual cell in so far as they bear upon our data. I especially refer to electron-microscopic studies of Sjöstrand[43,44] and DeRobertis[45,46] and to the histochemical measurements of Lowry and his associates.[47,48,49] In these histochemical measurements microsamples of tissues are taken from the various layers and even sublayers of the retina in frozen-dried sections and the samples analyzed by microtechniques (developed in conjunction with this particular work on retina and brain) for the activity of individual enzymes of respiration, glycolysis and other systems. Doctor Lowry was so kind to supply his recent data with interpretations for this symposium, but only a few of the enzymes measured will be mentioned specifically.[48]

Electronmicroscopy proved that the outer limb consists of a pile of discs with a diameter corresponding to the diameter of the limb. These discs are manifestations of protein layers alternating with layers of oriented lipid molecules. Lowry's measurements [47,48] indicate that the outer limb lacks the respiratory and glycolytic systems. It contains none of the essential enzymes of these systems; it also lacks the measured enzymes of the hexose monophosphate shunt.

In contrast, the inner limb appears to be equipped with a metabolic system of very high capacity: Firstly, it is extremely rich in malic dehydrogenase [47,48] and certain other enzymes of the respiratory system. Lactic acid dehydrogenase, on the other hand, is not higher than in other layers.[47,48] Secondly, the ellipsoid is very densely packed with mitochondria and essentially consists of nothing else but longitudinally arranged mitochondria.[43,44,45] It is now a well established fact that the mitochondrion is that cell particle which contains the enzymes of the respiratory system and the chain of electron carriers needed to reduce oxygen. No mitochondria were found in the inner limb outside of the ellipsoid body.[45] Moreover, the visual cell of the rabbit is said to lack mitochondria all over the cell down to its proximal end.[45] In the rat, however, an additional single mitochondrion lies in the terminal rod fiber close to the synaptic ending.[50] From the electronmicroscopic evidence, we may assume that the respiratory system of the rabbit's visual cell is contained exclusively by one small part of the cell (ellipsoid body) and that all other parts of the visual cell probably lack the ability to consume oxygen, and to derive energy from respiration. In species which have a single mitochondrion close to the synapse, the same statement would apply for at least the nuclear region of the cell. The enzyme measurements of Lowry et al.[47,48] do not completely agree with this conclusion. In the rabbit, malic dehydrogenase was relatively high in the outer nuclear and the outer plexiform layer.[47] However, in the monkey, the outer nuclear layer was very low in malic dehydrogenase while the synaptic layer was at a similar level as the inner layers.[47,48]

The region of the synaptic terminals has the highest level of lactic acid dehydrogenase of the monkey's retina.[47,48] In the same region glyceraldehyde phosphate dehydrogenase has its peak values and is twice that of the adjacent layers.[49] It seems that in this part of the cell metabolic conditions are the reversed of those in the inner limb. A very high respiratory capacity must reside in the latter while glycolytic capacity appears to be rather low. At the other end of the cell, the glycolytic capacity is probably the highest of all retinal layers but respiratory capacity is relatively low.

These findings make it abundantly clear that the striking division of the visual cell into different anatomical parts is also a division in metabolic properties. The visual cell seems divided into separate metabolic compartments which are exceptional in one or the other respect. One compartment, the outer limb, appears to lack the ordinary metabolic machineries completely; another compartment, the ellipsoid body, contains all or most of the cell's respiratory system in an exceptionally concentrated form; and still another compartment, the proximal region of the cell, is exceptionally well equipped to produce lactic acid. Nevertheless, these are parts of one cell. The following sections of this review will indicate certain limitations which this organization may impose upon cell life.

The unusual metabolic organization suggests that metabolic reactions participate in a direct manner with processes of visual cell excitation. Particularly one might ask whether the peculiarities in the metabolic organization at the ends of the cell have anything to do with the initiation of excitation (distal end) and with the transmission of excitation to the next neuron (proximal end). In regard to the proximal end, one can be very sure that a chemical system serves the transmission of excitation from visual cell to bipolar cell, just as in other

synapses. The structure of this region has been studied and beautifully illustrated by De Robertis.[48] The expanded proximal end of the visual cell is filled with vesicles which are a typical component of other synapses and which are believed to contain the synaptic transmitter. Nothing is known of the chemical nature of the transmitter at this synapse between visual cell and bipolar cell, but it is probably not acetylcholine.[51] Lowry's recent data [48] show that this region is amazingly rich in glucose-6-phosphate-dehydrogenase (in monkey *and* rabbit) suggesting that this enzyme and its cofactor, TPN, may be a part of the synaptic transmittor system. This enzyme is also high in the inner limb but less so than in the synaptic region.

When calculated relative to average brain tissue on a fat-free dry weight basis, glucose-6-phosphate dehydrogenase (G6DH) was found by Doctor Lowry to be 37 times higher in the synaptic region between visual cells and the bipolar cells than in the brain. In the region of the synapses between bipolar cells and ganglion cells this enzyme has approximately the same level as the average brain; in other words, G6DH is about 40 times higher in the outer synaptic region (outer plexiform layer) than it is in the inner synaptic region (inner plexiform layer). In the inner limbs the enzyme is likewise relatively high as much as 14 times the average brain; but throughout the bipolar cell and ganglion cell layers it is at the same or at a lower level than in the brain. 6-phospho-gluconate dehydrogenase, another enzyme of the oxidative shunt, has a similar pattern of retinal distribution as G6DH but it is less markedly increased in outer synaptic layer and inner limb in relation to the brain than is G6DH.

Of the many findings of Doctor Lowry, the following should in addition be mentioned: hexokinase activity is high in the inner limbs, 2 times higher than in brain, but surprisingly low in outer nuclear and outer synaptic layer. In the outer synaptic layer its level is ⅓ that of the brain and in the outer nuclear layer it is a mere 7% of the brain value. In view of the high capacity of the outer synaptic region for glycolysis and oxidative shunt activity Doctor Lowry considers the possibility that glucose entering the retina from the choriodal capillaries is converted in the inner limbs to glucose-6-phosphate and diffuses as such to the other end of the visual cell. Such a relationship between the metabolic activities of inner limb and outer synaptic region could provide a unique mechanism for the control of retinal excitation. Certainly, Doctor Lowry's contributions will greatly influence future retinal research whatever its subject may be.

Visual Cell Death from Poisons

A high degree of functional and structural differentiation necessarily imposes limitations upon the capacity of a cell to survive impairment of its metabolism. The paucity of visual cell pathology—except for hereditary conditions—would suggest that the visual cell despite its high and unusual differentiation evolved a much greater capacity to withstand adverse environmental conditions than brain cells or even the retinal ganglion cells and the optic nerve. The high resistance of the retina to anoxia or ischemia particularly would point this out. Wegner,[52] found that ischemia of the human eye does not produce irreversible retinal damage unless extended for more than 30 minutes. Complete recovery of the rabbit's visual cells occurs even when ischemia is imposed for 60-75 minutes.[53] Retinal ganglion cells and bipolar cells in the rat degenerate after 20 minutes of ischemia whereas visual cell death requires about twice as long a period of ischemia.[54,55] However, these findings only indicate a high capacity of the visual cells to survive lack of oxygen a condition to which brain cells are particularly vulnerable.

It was a surprise, nevertheless, when the visual cells were found to be destroyed in the rabbit after intravenous iodoacetate in doses which were well tolerated by the animal as a whole. Moreover, the destruction

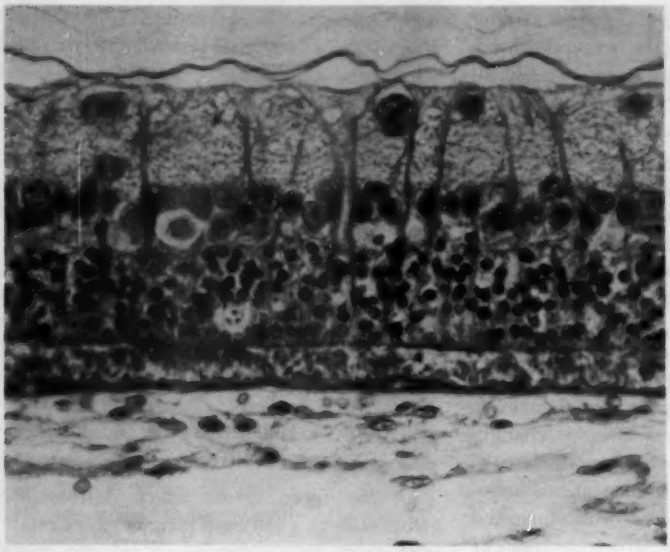


Fig. 11. — Pyknosis of the outer nuclear layer 48 hours after one-time exposure to x-radiation. The illustrated retinal regions received approximately 7000 r. (250 kv.). Section shows that the inner layers are preserved whereas all nuclei of the outer nuclear layer are deteriorating; outer and inner limbs are likewise disappearing the ellipsoid bodies apparently resisting the lytic process most.

of the visual cells was selective in the sense that the other cells of the retina (bipolar cells, ganglion cells, pigment epithelium) were undamaged morphologically unless the doses were in excess of those needed to destroy a large fraction of the visual cells. In the course of these studies, two other agents—oxygen poisoning and x-radiation—were found to have the same selective effect upon the visual cells although their mechanism of action probably differs from that of iodoacetate. Hence, the high degree of differentiation and the specific metabolic organization of the visual cell seems, as expected, to be associated with particular vulnerabilities of the cell. It will be shown below that the selective vulnerability to iodoacetate and oxygen poisoning becomes manifest as the cell develops to its full functional capacity.

The histological phenomena of visual cell death are the same for all three agents.[35] Pyknosis was the first sign that the cell had been damaged beyond repair (Fig. 11). Invariably, pyknosis was followed within one or two days by chromatolysis; the basophilia diminished progressively inside the pyknotic mass while a nuclear shell remained; later this shell broke into fragments and dissolved. Simultaneously with the nuclear changes, inner and outer limbs underwent swelling, fragmentation, homogenization and dissolution. Whatever the end products of this disintegration may have been, they were rapidly absorbed so that 3 to 6 days after the poisons no trace of visual cell remained, not even a fluid filled space.[6,7,35] No other tissue replaced the missing layers of the retina.

The visual cells of the central region of the rabbit were the most susceptible to iodoacetate and oxygen poisoning. Susceptibility decreased from center to periphery in a manner very similar to the progress of postnatal visual cell differentiation. Curiously this situation was reversed for x-radiation in so far as the dose needed to destroy the central cells exceeded that which killed the majority of the visual cells.

Similarities between retinitis pigmentosa and the effects of these poisons in the rabbit prompted us to study the monkey in order to determine whether cone cells differed in their susceptibility from rod cells. Oxygen poisoning in the rhesus monkey failed to produce retinal pathology prior to fatal pulmonary involvement. However, iodoacetate and x-radiation affected the visual cells of

the rhesus monkey in much the same manner as in the rabbit with the exception that visual cell death primarily or exclusively involved the rod cells.[6,7,8,56] The greater resistance of the cone cells was most strikingly evident by the completely normal appearance of the foveal cones when iodoacetate or x-radiation had destroyed all rod cells in the vicinity of the fovea and over large peripheral regions. It was possible to destroy (by iodoacetate) all rod cells except for a few at the ora serrata while the cones of the fovea still remained undamaged for nine months at least.[7] Obviously these cones were functioning and enabled the monkey to see. Outside of the fovea atrophic changes reduced or destroyed cone function, but the cone cells as such survived. The changes they underwent consisted of broadening and shortening to complete disappearance of outer and inner limb (with the ellipsoid body moving into paranuclear position). They were strictly related to the disappearance of the rods and increased in severity from the fovea through the parafoveal region as the distance between individual cones becomes greater.[6,7] Their cause is probably the lack of mechanical support by the rods which exposes the cones to the pressures which hold the retina against the pigment epithelium.

These findings clearly demonstrate that the monkey's cone cells are less vulnerable to iodoacetate and x-radiation than the rod cells. Since these two agents probably produce rod cell death by different mechanisms of action, the cone cell seems in a general way to be better safeguarded against irreversible damage than the rod cell. Either its metabolic organization is such that these poisons have less chance to kill the cell or its capacity for the rapid repair of a severe damage is a greater one. For the same reason, a genetic visual cell defect may spare the cone cell merely because this cell has a greater capacity to compensate for such defect.

Many phenomena of the iodoacetate effect in rhesus monkey, rabbit or cat are strikingly the same as in retinitis pigmentosa. Practically all essential histological and most ophthalmoscopical signs of the disease are simulated by iodoacetate with the exception of the typical pigmentary changes. On the basis of this similarity it was suggested that the genetic abnormality and the iodoacetate effect might involve the same metabolic system, the genetic abnormality affecting one enzyme or a cofactor of glycolysis.[7] The likelihood of this specific possibility somewhat suffered when the other agents were found to produce the same or similar pathology as iodoacetate.

Phenomena Related to the Iodoacetate Effect

Of the three agents which destroy the visual cells in a selective manner, iodoacetate is the only one about which we can make definite assumptions as to the basic mechanism of its action. This poison was introduced by Lundsgaard as a very effective and apparently selective blocking agent of glycolysis in the frog's muscle. Since then the agent has been widely used as a tool of physiologists to estimate the participation of anaerobic energy in the maintenance of cell function. There is overwhelming evidence that the glycolytic inhibition by iodoacetate is due to inactivation of triosephosphate dehydrogenase with which iodoacetate more readily reacts than with most other SH-enzymes.[57,58]

In order to show that the *in-vivo* effects of a blocking agent is caused by the inhibition of the enzyme system in question it must be proven that the system is actually inhibited *in-vivo* and that a quantitative relationship exists between the biological effect and the inhibition of the system. It was found that intravenous doses of iodoacetate which just blocked retinal excitation decreased lactic acid accumulation in the retina at least 30-50% during a subsequent period of ischemia.[7] Furthermore, rabbit retinas removed one half hour after intravenous injection of a dose known to damage irreversibly all visual cell produced 70%

less lactic acid under aerobic condition in the Warburg vessel than control retinas.[35] Circumstantial evidence that iodoacetate produces its effect by the inhibition of glycolysis was obtained by comparison of the effectiveness of anoxia and iodoacetate in different species. For instance, the ERG of frogs was found very insensitive to iodoacetate during air breathing but it disappeared almost immediately when subsequently the blood circulation was stopped, although its anoxia resistance in the absence of iodoacetate was actually very high. In agreement with this, aerobic glycolysis of the frog's retina is known to be low and its Pasteur effect to be high. Furthermore, ERG components of rabbit and cat [5,7] which responded very rapidly to anoxia resisted iodoacetate and vice versa those rapidly affected by iodoacetate resisted anoxia—a relationship to be expected if iodoacetate impairs functions depending mainly upon glycolysis.

On the basis of these findings the effect of iodoacetate appears adequately explained by inhibition of glycolysis. The histochemical observations referred to above strongly support this assumption. The cytocidal effectiveness of iodoacetate in conjunction with the high capacity of the visual cells to survive anoxia therefore implies that a vital function or a vital region of the visual cell derives its energy principally from anaerobic reactions through the Embden-Meyerhof scheme.

Glycolysis is also high in the inner layers of the rabbit but these layers resist the cytocidal effect of iodoacetate. Since we have no reason to assume that glycolysis is less inhibited in these layers than in the visual cells, their greater resistance may mainly be caused by the close association of the glycolytic systems with a respiratory system which continues to be active to the extent that cell death does not occur. The separation in the visual cell of areas predominantly utilizing glycolysis from areas where respiration resides probably prevents this cell from utilizing respiratory energy at a vital site. It may be said that the visual cell due to its particular metabolic organization is almost as sensitive to impairment of glycolysis as is the brain cell to lack of oxygen. This is particularly pointed out by the rapidity of action of iodoacetate. The histological signs of cell death required 6 to 12 hours for their manifestations. However, the ERG changed in rabbit and cat in less than 20 seconds after the injection and it disappeared within 2-5 minutes.

The rapid effects of iodoacetate on the ERG indicate that the excitatory function of the visual cell is at least as much dependent upon glycolysis as is cell viability. Indeed, the block of the excitatory function seems to occur from less inhibition of glycolysis than the arrest of cell life. Furthermore unless the dose of iodoacetate exceeds that needed to abolish the ERG, the visual cells recover.

Details of the relationships between ERG change and iodoacetate effect are complicated by remarkable species differences which prevent generalization as to the most sensitive cell sites.[5] The b-wave which in the rabbit is most sensitive to iodoacetate, is very sensitive to anoxia in cat, monkey and rat. In the rabbit the a-wave disappears slowly (i. e. in several minutes) after the b-wave has been eliminated by iodoacetate whereas in the other species a- and b-wave disappear almost simultaneously and very rapidly. Evidently these differences concern the order of susceptibility of the functional components which the ERG measures. They suggest differences in the metabolic organization of the visual cell over the mammalian scale which may well be associated with structural or enzyme differences.

The great dependence of the visual cells on glycolysis necessitates ample supply of glucose. Papst [59,60] recently showed that the recovery of the ERG from hypoglycemia was 2-3 times slower than that of the EEG. Anoxia superimposed upon hypoglycemia also was much more effective than under control conditions. The glycogen reserves of the visual cell are probably high as indicated by the long survival time of the ERG during anoxia. The adult retina, moreover, continues to glycolyze in the

absence of glucose for about forty minutes under anaerobic conditions. Significantly in the young retina at 2 to 7 days this ability is much less, lactic acid production practically ceasing after 10 minutes.

Effects of Oxygen Poisoning and X-Radiation

The high susceptibility of the rabbit's visual cells to oxygen poisoning is a very remarkable phenomenon because like the iodoacetate effect it is indicative of an exceptional property of these cells. Visual cell death occurred during exposure to oxygen concentrations as low as 60% at ambient pressure (5 day exposure). 90-100% oxygen at ambient pressure destroyed the majority of the visual cells within 40-48 hours.

Cell death is an unusual phenomenon of oxygen poisoning. It has been described so far only for the spermatogenic epithelium.[61] Furthermore, cancer growth and viability in animals are said to be affected (see Ref. 62), and Warburg[63] recently reported that Ehrlich's ascites cells show depressed glycolysis and viability after long *in-vitro* exposure to oxygen pressures higher than 2½ atm. It may not be mere coincidence that visual cells resemble cancer and spermatogenic cells in susceptibility to oxygen. The spermatogenic cell according to Karli[64] is also sensitive to iodoacetate, and cancer tissue has, like the retina, a very active glycolytic system. However, we have no evidence as yet that oxygen poisoning affects the visual cells by inhibition of glycolysis. A significant summation of the effects of iodoacetate and oxygen poisoning was not observed in tests performed to check this possibility.

At high pressures of oxygen the b-wave was selectively and very rapidly affected, whereas high oxygen concentrations at ambient pressure diminished the amplitude of all ERG components more or less simultaneously and slowly. At 1 atm. the change began to appear after 15 hours and slowly progressed during the following 24 hours. Recovery was limited to that fraction of the ERG which had been reduced for less than 8 hours. Hence, it seems that once the cells have ceased to contribute to the ERG their life processes are irreversibly affected within a few hours unless oxygen exposure is terminated. Because of the slow progress of ERG failure and of visual cell death, oxygen poisoning at ambient pressure simulated the course of hereditary visual cell degeneration in the young mouse to a remarkable degree.

Gerschman, et al.[65] suggested that oxygen poisoning has a similar basic mechanism of action as x-radiation and that high concentrations of oxygen result in the formation of oxidizing free radicals in the tissue. Effects of these two agents on the visual cells showed many similarities but they differed also in important aspects. X-radiation much more than oxygen affected the vital function of the visual cell whereas oxygen had first of all a reversible effect upon ERG and retinal excitation.

The most outstanding properties of the action of x-radiation on the visual cells[66,67] were (1) a very close association of effects upon the ERG with those upon cell life, (2) the acuteness of cell death when a critical dose level had been surpassed and (3) a great capacity of the visual cell to recover from latent effects of x-radiation so that fractionation into daily doses increased the cytocidal dose very strikingly. The lethal cell dose (about 5000 r. at 250 kv. with single exposure) was only 10-20% higher than the dose which produced any ERG effect at all. To be immediately or rapidly effective upon the ERG, doses higher than the cytocidal ones had to be applied. Cell death was very acute, pyknosis developing never later than 18-24 hours after radiation. X-radiation, therefore, appears to select for the site of its action a very vital system of the visual cell. In this it differs from all the other poisons we studied. Its action suggests that there is within the visual cell a critical center or a very critical system, the impairment of which

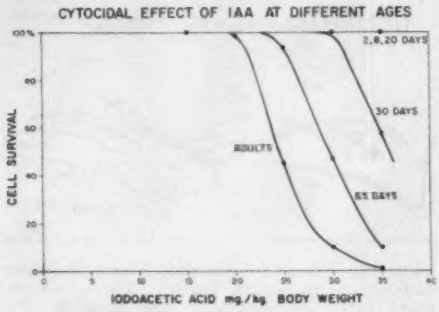


Fig. 12.—Effects of iodoacetate at different ages. The graph relates the dose administered to the extent of visual cell survival in a retinal arc from optic nerve through ventral region to ora serrata. It shows that cell death did not result from doses up to 35 mg/kg. at ages of 2 to 20 days. The effectiveness of iodoacetate was still reduced at 65 days compared to the adult.

results in the almost simultaneous loss of cell life and sensory function.

Changes with Age in Cytocidal Effects

In order to establish a relationship between visual cell differentiation and susceptibility to cytocidal poisons, the effectiveness of iodoacetate and oxygen was tested at various ages. The extent of visual cell death produced by these agents at different dose levels was measured through the ages of 2 days to 65 days and compared with the effects in the adult animal.

Iodoacetate was tested in doses covering the range of from 15 mg. per kg. to 35 mg. per kg. bodyweight. Except for 15 mg/kg., the dose was divided into two injections 6 hours apart. The first injection was always 15 mg/kg. Injections were intracardiac in the youngest age groups (2-10 days), intravenous in the others. The systemic toxicity of iodoacetate was in the young animals much greater than in the adult; a dose of 40 mg/kg. was almost 100% lethal at 2 to 10 days; rarely did an adult animal succumb to this dose. Oxygen, however, affected the young animals much less than the adult.

As shown in Figure 12, 20 mg/kg. of iodoacetate was needed in the adult to produce minimal extent of visual cell death and 35 mg/kg. sufficed to destroy almost all cells (the plots of "cell survival" in Fig. 12 and 13 apply to a retinal arc from optic nerve to ora serrata through the ventral segment of the retina which is the most susceptible segment). At 2, 8, and 20 days all visual cells in each animal survived doses of iodoacetate up to the highest tested. After 20 days the effectiveness of iodoacetate became apparent and increased slowly with age as shown in Figure 12. It is noteworthy that at 65 days the susceptibility to iodoacetate was still less than in the adult.

Similar results were obtained with oxygen poisoning. With 2-5 days old rabbits, even exposure for 72 hours to 100% oxygen at ambient pressure killed only few visual cells, whereas in the adult 30 hours of oxygen breathing sufficed to produce widespread visual cell death. Exposure of the adult to 1⅔ atmosphere of oxygen produced the death of almost all visual cells within 24 hours, but the young animals (3 to 5 days) exposed for 30 hours to the same pressure showed only occasionally visual cell death, in a restricted area. At 20 days

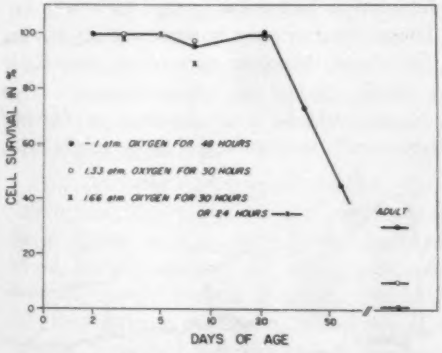

CELL SURVIVAL IN %
1 atm. OXYGEN FOR 48 HOURS
1.33 atm. OXYGEN FOR 30 HOURS
1.66 atm. OXYGEN FOR 30 HOURS OR 24 HOURS
ADULT
DAYS OF AGE

Fig. 13.—Cytocidal effectiveness of oxygen at different ages. The evaluation of the extent of visual cell death is the same as in fig. 12. The age in days (abscissa) is plotted on logarithmic scale. Maximal effect was obtained in adult animals by exposure to 1⅔ atmospheres of oxygen for 24 hours; at age of 2 to 5 days exposure to the same pressure for 30 hours was still ineffective. Pulmonary pathology developed during exposure of adult rabbits to 1 atm. for 48 hours; none existed in the young ones (2 to 40 days). If pulmonary pathology had been absent in the adult, visual death from 1 atm. probably would have been more extensive.

oxygen was also ineffective at pressures which had very striking results in the adult (see Figure 13). Curiously at 6 to 8 days the effectiveness of oxygen poisoning seemed to be increased compared to the age of 5 days and 20 days.

It is difficult to conceive that these striking changes with age result from differences in choroidal blood supply or tissue permeability. Especially with respect to oxygen poisoning such explanation seems most unlikely because the high oxygen pressures tested at the young age should have become effective even if choroidal blood flow differed from the adult. We conclude, therefore, that visual cell susceptibility to these poisons is less in the young than in the adult because of differences in visual cell properties.

If the selective susceptibility of the adult visual cells is, as we believe, related to the differentiation into separate metabolic compartments (see above), then it is not surprising that the immature visual cell escaped cytocidal effects of these poisons. However, susceptibility increased throughout the late phase of ERG development when this differentiation had already progressed very far. The striking increase in susceptibility during this phase, therefore may result from only a subtle change in cell properties. This change could be a consequence of further functional development, but it might also

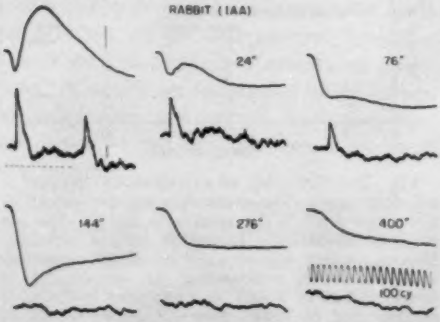


Fig. 14.—Effect of intravenous iodoacetate (15 mg/kg.) on ERG and optic tract potentials in the adult rabbit. Numbers beside tracings denote times in seconds after the start of injection. The light stimulus is a tungsten light flash of 120 msec. duration. (From Noell ref. 5).

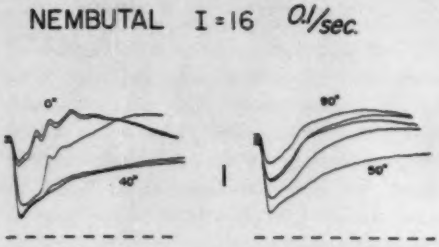


Fig. 15.—Effect of intravenous Nembutal (pentobarbital) in the rabbit (adult). The eye is exposed every 10 seconds to the stimulus. The recording film is moved in the camera when 5 successive responses have been photographed.

be related to the dissipation of synthetic capacities which the growing cell possesses.

ERG Phenomena from Effects of Poisons

Observations on the ERG under the influence of poisons have played in the past a major role in attempts to analyze and define its main components.[68,69] The interest of our studies on the ERG effects of poisons mainly rests on the possibility that these effects might reveal aspects of the metabolic and specific biochemical organization of retinal function. The effect of iodoacetate on a- and b-waves, and the striking changes in c-wave and d. c. potential caused by azide[7,70] are instructive examples of the usefulness of the ERG in providing information on these points. However, the ERG changes *per se* may serve as guides for the ultimate decoding of the ERG in terms of retinal excitation. In the following the major changes of a- and b-waves which were observed under the influence of poisons will be described briefly.

Selective b-wave depression.—Since the earliest days of electroretinography it has been known that the elimination of one component may unmask another component with which it coincides. A very striking example of such an effect is the selective elimination or depression of the b-wave. As shown for iodoacetate (Fig. 14) and Nembutal (Fig. 15) this effect results in the emergence of a high amplitude a-wave. Apparently the poisons delay the appearance of the b-wave and slow its de-

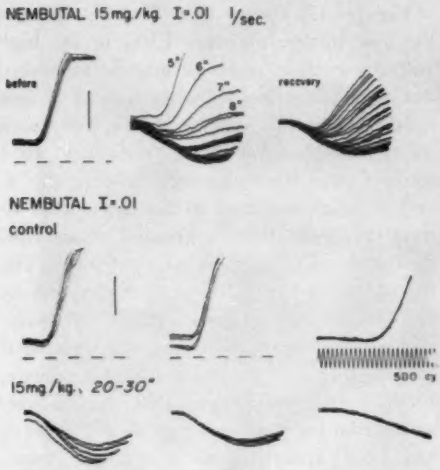


Fig. 16.—Effects of Nembutal on scotopic ERG provoked with low stimulus intensity. Stimulus rate is one per second. Center picture of upper row shows the rapid failure of the b-wave, beginning 5 seconds after the injection and leading to the emergence of a cornea-negative potential of shorter latency than the b-wave it replaces. The film is moved to a new frame (right picture) about 30 seconds after the injection. Recovery of the b-wave proceeds steadily, consisting of the progressive speed-up of the potential. In consequence, the cornea-negative wave becomes progressively curtailed and flattened. The two lower rows show the effects of Nembutal in the same animal after 2 further injections (left and center pictures). The pictures to the right are obtained from a different animal.

velopment so that with each additional slowing or delay of the b-wave a further fraction of the a-wave appears. The reverse occurs during b-wave recovery provided the effect is very transient and has not led to a-wave involvement.

The slowing in the development of the b-wave is best displayed by responses to low intensity stimuli. In Figure 16 the action of Nembutal is shown to increase the latency, decrease the slope and reduce the amplitude of the b-wave. As these changes proceed a cornea-negative potential develops although the control response was devoid or almost so of any sign of a-wave activity. Furthermore, the a-wave which appears during the elimination of the b-wave has a shorter latency than the b-wave it replaces.

After b-wave elimination an a-wave appeared at all stimulus intensities during scotopic conditions, and all these a-waves had shorter latencies than the b-waves they replaced. Their latency was a function of stimulus intensity just as is b-wave latency. The effect suggests a curious relationship of a- and b-waves: the early portions of the a- and b-wave of the scotopic ERG may coincide and cancel each other, or b-wave slowing may accelerate a-wave appearance as if in a chain of chemical reactions, the block at one step leads to the accumulation of a reaction product at an earlier step of the chain.

Selective b-wave elimination was always associated with the disappearance of retinal excitation as measured by the optic nerve potentials (Fig. 14). The latency of these potentials increased 2 or 3 times when b-wave generation was slowed. Such increase in the latency of retinal excitation did not occur or to slight extent only, when a- and b-waves almost simultaneously diminished. The retardation of the optic nerve responses to the degree indicated, suggests that slow processes of retinal excitation undergo further slowing as a poison eliminates the b-wave in a selective manner. These slow processes of retinal excitation one expects to be localized in the visual cell. Hence, we may assume that the poisons which selectively retard b-wave and retinal excitation do so by interfering with a slow process of visual cell excitation.

A great variety of poisons produce selective b-wave depression. In our experiments these agents were of such diverse kinds as azide, barbiturates (of the sedating or convulsant type), carbon dioxide, trichloroethylene, oxygen poisoning, iodoacetate, and anoxia. Species differences existed for iodoacetate and anoxia but were not found with azide and barbiturates. All these poisons may have in common effects upon general metabolism, and since selective b-wave depression is the most frequently obtained effect of metabolic poisons, we may infer that the slow processes of visual cell excitation are generally the least safeguarded against metabolic impairment.

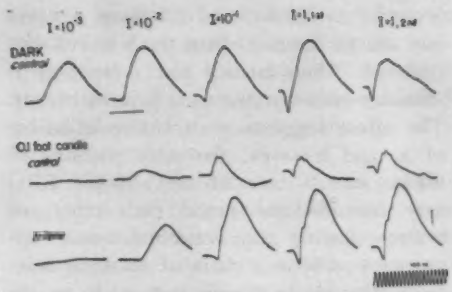


Fig. 17.—Effect of Trilene on photopic ERG of the rabbit. Curare and artificial respiration. The stimulus is a tungsten light flash of a duration of 120 msec. The upper row shows the scotopic ERG of the animal during the control period at different light intensities. The ERG marked "I=1,2nd" follows the preceding response (to its left) after 4 seconds; it is reduced in amplitude due to incomplete recovery from preceding flash. Middle row illustrates the control ERGs of the same animal during photopic condition. The ERGs of the bottom row are recorded during subsequent "Trilene" administration.

The rapidity with which selective b-wave elimination can be produced is very great. Azide, Nembutal or trichloroethylene affected the ERG within 15 seconds after administration. However, truly selective b-wave elimination was a very transient event. Either a-wave involvement followed rapidly or recovery occurred. With Nembutal, for instance the retinal effect already diminished while the effect upon the brain waves reached maximal extent. It may be that diffusion of these agents into and out of the retina is faster than for the brain, but one must also consider that rapid adjustments may occur within the visual cell in response to the inhibition of certain metabolic reactions.

ERG enhancement.—Some of the poisons (azide, Nembutal, trichloroethylene) which produced selective b-wave depression were found to enhance ERG amplitudes at lower doses. The most striking enhancement was observed with trichloroethylene ("Trilene") administered as a vapor to the inspiratory air. The agent is used clinically as an adjuvant to general anesthesia because of its analgetic property. Its ERG effects were unexpected and accidentally discovered during an investigation of brain wave effects.[10]

Figure 17 shows that Trilene changes the low voltage photopic ERG to the high voltage scotopic one. It was administered during a background illumination of .1 foot candle when the rabbit's ERG had taken its typical photopic form. Within ½ to 1 minute after the start of Trilene, the a- and b-waves increased to the extent that almost the same ERG appeared as recorded during the control, dark adapted state. The threshold of the ERG was unchanged or more often slightly increased. No significant increase or decrease in the d.-c. potential across the eye occurred simultaneously. Furthermore, the Trilene ERG lacked conspicuously the c-wave when a- and b-wave had been enhanced to a scotopic form. Trilene produced only slight enhancement when tested during complete dark adaptation with long duration stimuli.

The remarkable enhancement of a- and b-waves by Trilene illustrates that the change in visual cell responsiveness produced by the photopic condition is subject to reversal by chemical means. The effect of Trilene is probably best defined as one which "inhibits" the ability of certain parts of the ERG system to shift from scotopic operation to the photopic one.

Sodium azide and Nembutal had a similar effect as Trilene in the sense that they produced enhancement of a- and b-wave to the greatest extent under moderate photopic conditions. These two agents, more so than Trilene, also increased and broadened the b-wave of the scotopic ERG. Nembutal in addition produced the phenomenon of repetitive b-waves at low stimulus intensity during b-wave recovery from depression. All three agents enhanced the amplitude of the wavelets superimposed upon the rising phase of the b-wave. Trilene and azide, furthermore, increased transiently the optic nerve response to illumination as if the intensity of light stimulation had been increased, and azide, furthermore, increased or inhibited the activity of the ganglion cells in the dark depending upon the responsiveness of the particular element to light.[7]

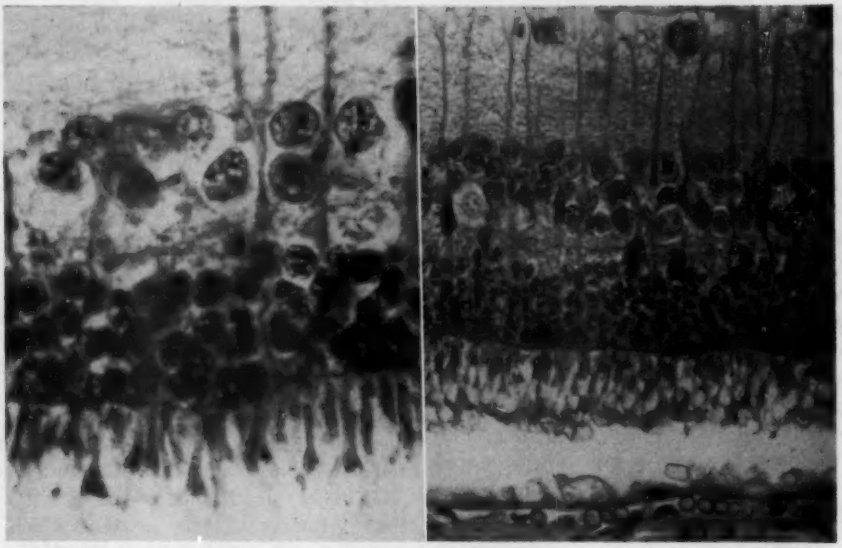


Fig. 18.—Retinas showing outer and inner limb degeneration resulting from exposure to x-radiation. At the left, some outer limbs have about normal form but most are affected; at the right, all outer limbs have disappeared. Note that Müller cells are moving into the outer nuclear layer. Hematoxylin-eosin. Left—Objective × 50; right—Objective × 97.

Depression of a- and b-waves.—It rarely occurred in our experiments that a metabolic poison primarily and acutely diminished both waves simultaneously. Almost without exception, the initial effect on one wave was greater than on the other, but acute preferential a-wave involvement was also of rare occurrence.[5,7]

Chronically, a simple depression of a- and b-waves was found after iodoacetate poisoning. Part of the retina had then lost its visual cells whereas in other parts no histological abnormality was evident. In these cases the amplitude reduction must be attributed to the loss of functional elements whereas preservation of the normal form of the ERG is explained by the normal histology of the cells remaining. An equivalent condition was encountered acutely during x-radiation of the eye. When the x-ray dose, necessary to destroy immediately visual cell function had been reached in one retinal area, the ERG merely changed in amplitude. A condition then seemed to exist where a part of the retina failed to contribute to the ERG whereas other parts still were functioning normally.

Degeneration of the Sensory Organelles

The sensory organelles are the main target of the degenerative process of which Figure 18 illustrates typical examples. If this degeneration is very advanced, the outer limbs have disappeared and the inner limbs have changed to such bizarre forms as shown in Fig. 18. If slight, the manifestations are poor staining of outer limbs and irregular elongation of the inner limbs. This degeneration resulted in our experiments from x-irradiation, oxygen poisoning at ambient pressure, and intravenous iodate. As one would expect it developed slowly and required at least 1-2 days for its manifestation. Tansley[71] and Johnson[72] described a similar pathology in vitamin A deficiency and called to attention a weakening at the junction between inner and outer limb. In our experiments this weakening was occasionally observed after iodate but not after oxygen poisoning or x-radiation.

Iodate was by far the most effective of the three agents, and the rod changes it produced rapidly progressed to the extreme. Iodate poisoning also resulted in widespread disappearance of the pigment epithelium. After oxygen and x-radiation the pigment epithelium was generally preserved. Oxygen produced degeneration only when administered at doses which resulted in the death of the visual cells of at least the central area; the surviving cells then showed the degeneration to a mild or moderate degree. Similarly, a significant degree of rod degeneration after a single dose x-radiation almost always was associated with cell death in one retinal area. When fractionated into daily doses, however, x-radiation produced exclusively widespread outer and inner limb degeneration, the visual cell as such escaping death.[67]

In previous discussions of the iodate effect [7,35] it was stressed that the rod abnormality might result from the loss of pigment epithelial function because of its great importance in the maintenance of retinal homeostasis. This still appears a valid explanation so far as iodate is concerned. However, it may not apply for the effects of oxygen and x-radiation since under these conditions rod degeneration occurred in the absence of a visible damage to the pigment epithelium. Here, direct effects upon the sensory organelle might be involved. It is noteworthy that iodoacetate generally failed to produce any signs of this particular abnormality.

This rod abnormality is of special interest because the visible damage of the sensory organelles is associated with specific ERG changes. A preferential reduction of the a-wave consistently was observed whenever any degree of rod abnormality had developed after oxygen poisoning or x-radiation. The b/a ratio, measured from responses to maximal stimuli, and used for correlating the ERG with histology, was increased, for instance, 3 times after x-irradiation which had resulted in exclusive rod degeneration. Such a-wave reduction could appear without a change in b-wave amplitude.

The most outstanding ERG abnormality which developed within a few days after injection of iodate was a lengthening of the latent periods of a- and b-waves. As shown in Figure 19 the ERG appears to have shifted on its time axis to the right. Simultaneously the ERG threshold is increased by several log units and ERG amplitudes are markedly reduced.

This ERG abnormality after iodate clearly differs from all those described under effects of metabolic poisons. It may be best accounted for by a lesion located close to the sites of the early events of excitation where the histological changes are also most severe. The major significance of these ERGs is their great resemblance to those which are recorded in the mouse afflicted with hereditary visual cell degeneration.

Hereditary Visual Cell Degeneration in the C3H Mouse

Keeler in 1924 described the hereditary lack of the visual cells in mice derived from the Bagg albino strain. He reported in several publications on various aspects of this condition and established that it is inherited as a recessive Mendelian character.[73-78] He described the abnormality as "rodless" and defined it as "characterized by the absence of the visual cells (rods) the external nuclear layer, and the external molecular layer." [75] The condition was sub-

Fig. 19.—Electroretinogram after iodate poisoning. Top row—controls; middle row—42 hours after intravenous injection of 5 cc. of 2% solution of sodium iodate; bottom row—3½ days after 4 cc. of 2% sodium iodate.

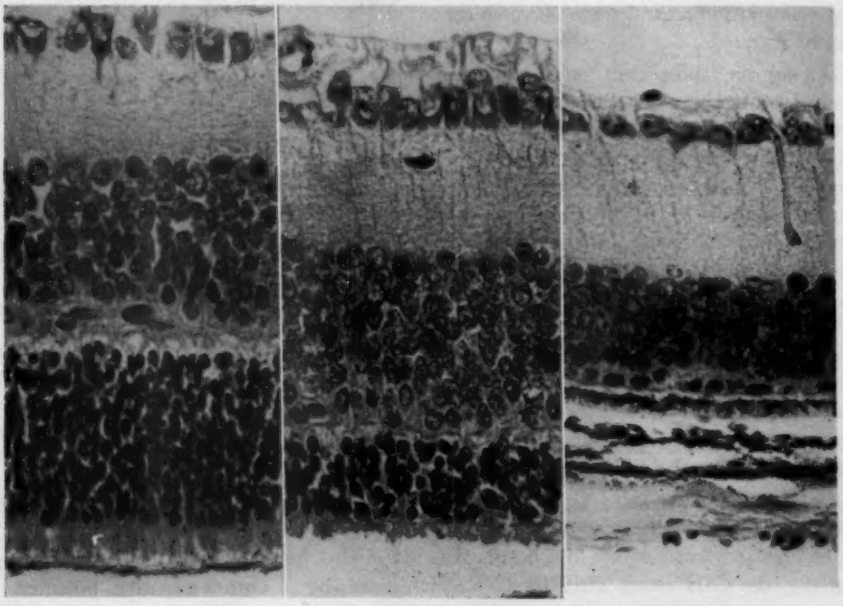
sequently found in various mouse colonies in this country and in Europe (see Ref. 79) but strains carrying the Keeler abnormality seem to have been lost in the following decades with the exception of two strains (BDB and P/JAX) which are difficult to breed. The interest in this condition was immediately revived when Brückner in 1951 reported on a similar hereditary retinal affection in albino mice at Basle.[80] He recognized the disorder ophthalmoscopically by the diminution in the number and the diameter of the retinal vessels. Histologically, there was a great reduction or loss of the visual cells. Extensive genetic and histological studies on the Brückner mice were subsequently undertaken by Tansley [81] and Sorsby et al.[19] Karli [64,82] investigated morphological and psychophysical aspects of the condition in comparison with the effects of iodoacetate upon the rabbit's retina.

Since the two strains with a hereditary visual defect availiable in this country are difficult to maintain we undertook to screen mouse strains [1] by the ERG under the assumption that the abnormality might well exist unrecognized since Keeler and Brückner discovered it accidentally. About 18% of Swiss mice were found to lack an ERG and to show histologically almost complete absence of the visual cells. The same condition was revealed (100%) in the pure line C3H mouse of all colonies examined. As we found out later, the C3H abnormality had been reported in 1954 by Dunn [83] after it had been discovered during an investigation of x-radiation effects on young retinas.

The C3H mouse is well pigmented and its eyes lack any overt abnormality. The mouse is alert and moves around with skill and perhaps particular grace. Its lack of vision is non-apparent. Since a well known, easily bred, pure line strain offers great advantages we performed our studies mostly on the C3H mouse. We established that the condition is autosomal recessive and

[1] I am indebted to Dr. Hauschka and Dr. DiPaolo for generous supply of mice.

Fig. 20.—Retina of the C3H mouse at age of 10 days (left), 14 days (center) and 25 days (right). Hematoxylin-eosin. Objective × 50.



that it probably does not differ genetically from that of our Swiss mice.

Histological development of the C3H abnormality (Fig. 20) was practically the same as described by Sorsby et al.[19] for the Brückner mice. No differences between C3H and control strains were evident at birth and for about 7 to 10 days thereafter. At 10 days the C3H and control retinas had advanced to a stage comparable to that of the 8-9 day old rabbit, i. e. the inner layers were well differentiated, the outer plexiform layer was established, the sensory organelles had grown to ¼ or ⅕ their adult length, and outer and inner limbs were clearly recognizable. However, some differences between C3H and control retinas were evident at this age: the outer nuclei of C3H were more densely stained and their chromatin more clumped; the outer nuclear layer was slightly reduced in width through the central region and the sensory organelles were more difficult to distinguish one from the other than in normal retinas. Moreover, within the central region of C3H a few nuclei were pyknotic. The death of visual cells had obviously begun. Simultaneously further growth of the sensory organelles in the central region became completely arrested.

During the subsequent days visual cell development progressed through the retinal periphery to the stage of a miniature rod. However, as development proceeded over the retina more and more visual cells died. At the age of 14 to 15 days the outer nuclear layer of most of the retina was reduced to about 3 rows of nuclei and at 17 to 18 days to one row. At about 20 days, degeneration had spared only the visual cells close to the ora serrata and these cells vanished within 7 more days. Thereafter, the retina uniformly consisted of the (unchanged) inner layers, one row of outer nuclei, outer limiting membrane and pigment epithelium. Several months later some localized atrophy of choroid, pigment epithelium and inner layers did occur. Essentially, the C3H retina after 18 days had

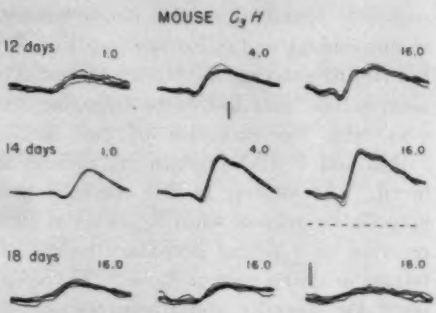


Fig. 21.—Electroretinogram of the C3H mouse at different age. Each age is represented by a different animal; records of bottom row are from three mice. Amplification is the same except for the right record of the bottom row.

the same appearance as that of the rabbit after iodoacetate or oxygen poisoning.

In breeding experiments, Keeler[76] succeeded in obtaining animals where the outer nuclear layer was reduced only to 3 or 6 rows instead of being absent almost completely. Outer and inner limbs of these cells were missing or dwarfed. A similar modification of the disease has been described by Sorsby et al.[19] So far, we have been unable to find (or to produce) this modification which understandably could be very valuable in the analysis of the disease.

Despite the close association of visual cell differentiation with visual cell death, the C3H mice could develop the ERG as early as control strains. The ERG even increased in amplitude up to 14 to 15 days while visual cell differentiation proceeded over the periphery. It then declined as cell death became overwhelming and it disappeared between 20-28 days when the last cells at the ora serrata deteriorated.

The C3H ERG is abnormal in a typical manner almost as soon as it makes its first appearance: 1—Throughout the age from 12 to 18 days the C3H ERG (Fig. 21) is low in a-wave amplitude compared to the ERG of a normal control strain (Fig. 22) whereas its maximal b-wave amplitude approaches control size. 2—The b-wave of the C3H mouse arises very late after the stimulus but because it is immediately preceded by a cornea-negative potential this

late ERG differs strikingly from the normal slow scotopic ERG at low stimulus intensity. 3—The responsiveness of the C3H ERG is reduced so that the minimally effective stimuli are at 12 days already about 100 times higher than in controls.

These differences in the ERG of the C3H mouse compared to normal strains are not the result of either delayed development or arrested development nor can they be due to the increasingly smaller number of elements which contribute to the ERG in comparison to a normally differentiating retina. The ERGs of the C3H mouse differ distinctly in form from those recorded during

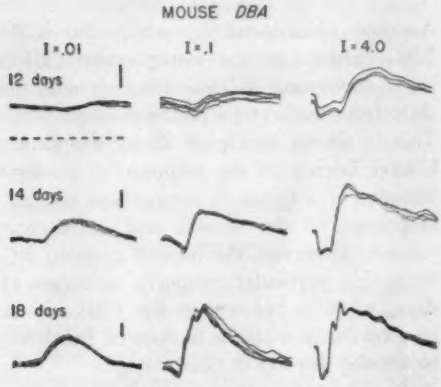


Fig. 22.—Electroretinogram of the normal mouse (DBA). Each age is represented by a different animal. Experimental conditions are the same as for the C3H mouse of figure 21. However the stimulus intensities cover a lower range.

a normal progress of retinal or ERG development. However, they are very similar to the ERGs of the rabbit after iodate poisoning during degeneration of the sensory organelles.

The ERG abnormality of the C3H mouse is occasionally of slight degree at the very beginning of retinal function. Figure 23 compares early ERGs of C3H mice and C57 Black mice of the same age. The illustrated C3H ERGs were the least abnormal ones we recorded from any litter; C57 Black was chosen because ERG development of these mice was generally slower than in other control strains. Compared on this

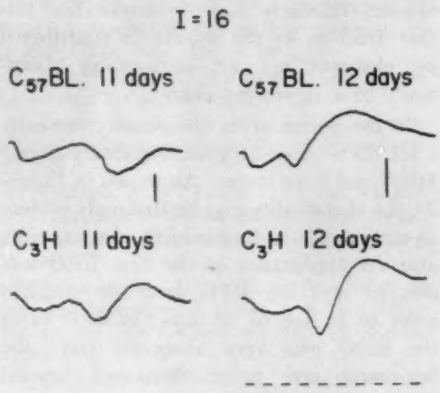


Fig. 23.—Early electroretinograms of the C3H mouse compared with the C57 Black mouse. The records at 12 days were obtained from litter mates of those tested at 11 days. C57 Black is a normal strain.

basis the abnormality of the C3H ERG is very slight indeed, but it can nevertheless be detected by the slow slope of the a-wave and (at 12 days) also by the delay in b-wave generation. It is noteworthy that the a-wave latency of these early ERG's (C3H) is not prolonged at 11 days or 12 days. Similarly one notes in Figure 22 that despite the prolongation of b-wave latency, the a-wave

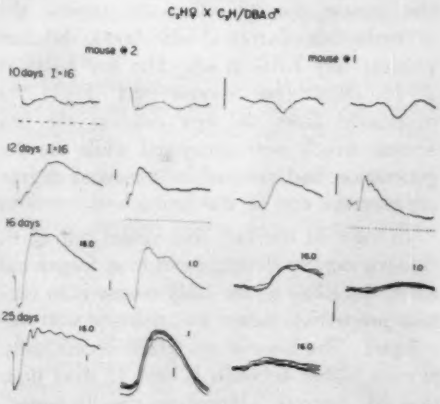


Fig. 24.—Electroretinograms of two litter mates from a cross between C3H and C3H/DBA. Mouse #1 was affected, mouse #2 did not differ from controls (DBA). The differences in the ERG of these animals are clearly evident as early as 10 days. The retina of mouse #1 at 25 days had the same appearance as illustrated in fig. 20 for this age. Visual cells showing small outer and inner limbs were present only at the very edge of the ora serrata.

appears relatively fast. I assume that this fast fraction of the a-wave is contributed by elements not yet performing abnormally to a significant degree.

In the degree of its abnormality the early C3H ERG showed a great variability among litters and litter mates. As shown in Figure 24, the abnormality may be strikingly evident as early as 10 days after birth. Occasionally also the appearance of the first ERG was delayed and the ERG was not obtained prior to an age of 12 days; in these cases the ERG was very abnormal from the beginning and never developed beyond minimal amplitudes.

Experiments as illustrated in Figure 24 enable an especially adequate comparison between the ERGs of affected and unaffected animals because both are contained in the same litter. In the experiment illustrated, a male C3H/DBA hybrid was bred with a C3H female. When the litter was tested at 10 days, the ERG already separated affected from unaffected animals. Those which later showed no signs of a histological abnormality had at all times an ERG which did not differ from that of normal control strains; those manifesting the disease showed either the typical abnormality or (after 20-28 days) did not produce any ERG at all. The low ERG at 20-25 days (see mouse #1 Fig. 24) originated from the few cells at the ora serrata which were preserved while the degeneration had reached its maximal degree all over the rest of the retina.

In view of the fact that visual cell death follows rapidly development to a functional state, the ERG of the C3H mouse is in certain properties rather surprisingly well developed. The b-wave amplitude occasionally is even higher between 12 and 15 days than that of controls. Moreover the "wavelet" which is interposed in the young ERG between a- and b-wave (see Fig. 23; compare also with Fig. 5 of young rabbit) is of normal size and similarly the wavelets superimposed upon the b-wave after further development are well evident (Fig. 25).

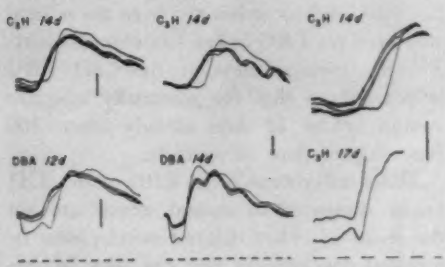


Fig. 25.—Responses to 1 per second flicker in C3H and DBA mice photographed by single frame exposures. The response which deviates from the others in b-wave appearance or in a-wave amplitude is the response to the first flash of the flicker. The ERGs at the right are recorded with faster sweep speed; right bottom picture shows the first (long latency) and the fifth (short latency) response to the flicker.

Another phenomena in which the C3H ERG behaves as the young control ERG is the shortening of b-wave latency with the shift from the scotopic to the photopic state. This is shown in Figure 25 by the longer b-wave latency of the response to the first stimulus of a flicker in comparison with the responses to the second and subsequent stimuli. However, the normal mouse ERG loses this particular property at about 14 days, while it persists in the C3H mouse in accordance with the failure of the ERG to develop beyond the early stages.

Since the characteristic ERG abnormalities of the C3H mouse resemble those of the rabbit during sensory organelle degeneration (iodate effect) we assume that these abnormalities are manifestation of abnormal sensory organelle function. The ERG signs themselves would point this out because they indicate a lesion or lesions early in the chain of reactions which leads to retinal excitation in response to light. The sensory organelle may develop abnormally from the very beginning or the visual cell may become incapable of maintaining functional and structural integrity of the growing sensory organelle. The fact, however, that sensory function appeared at all shows that the visual cell was able to develop all structures and processes needed for visual excitation. The subsequent death of the cell

may be interpreted as the final consequence of a deficiency which manifested itself first in the inability to support the growth of the sensory organelle.

The close association of cell differentiation with cell death suggests that this deficiency—if it existed prior to differentiation—became crucial as the cell underwent the biochemical changes associated with differentiation, or that it arose during differentiation by the failure to develop a system needed to maintain cell life once the cell had departed from its immature state.

Experimental attempts to analyze this deficiency and to determine its character will have to take into account all biochemical and physiological properties of the visual cell including those related to the photochemical system. Although the primary defect may be different in human retinitis pigmentosa, this analysis should lead to new approaches to the study of the human disease.

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DISCUSSION

CAPTAIN H. G. WAGNER, Naval Med. Res. Inst.: "The resistance of the retina to x-radiation has been sort of legendary so that this report came as a surprise.

It so happened that, at the time we first heard about it we had our hands on a patient who had received some 8000 r to one orbit. The radiation, however, had been fractionated over a period of time. We did look at his dark adaptation, but noticed no change between the two eyes or between his response and that of ourselves which we considered normal.

I was wondering, have you any clinical evidence along this line? Also, another thought was in regard to a similar effect of oxygen toxicity in humans."

DR. NOELL: "There are considerable species differences in the effectiveness of these poisons. Iodoacetate for instance requires much higher doses in monkey and rat than in cat or rabbit. I personally have no data on the effects in man of x-radiation or oxygen-poisoning. Oxygen poisoning is well known to produce constriction of the visual field in man and this is said to be one of the most consistent signs of oxygen poisoning at 2-3 atmospheres. If x-radiation removes or reduces the electroretinogram in the rabbit this is almost always irreversible and due to either death or degeneration of the outer limbs. Oxygen poisoning is a reversible phenomenon; exposure must be extended for several hours after the cell lost function in order to produce cell death. Otherwise, the cell recovers. The dose of x-radiation which affects the rabbit's ERG is very high, generally over 5000 r, and fractionation reduces the effectiveness of x-radiation upon visual cell life very markedly. X-radiation is more effective, curiously, at 2000 kv. than at 250 kv. and in turn 250 kv. is apparently more effective than 100 kv. I think that, unless in man radiation is given in one high dose visual cell death will not occur. But I could not guess what the lowest dose level might be which produces the degeneration of the outer limbs."

DR. JERRY HART JACOBSON, New York Eye & Ear Infirmary: "I would like to know, Dr. Noell, if at any point you have taken iodo-acetate and put it on the surface of the exposed or excised retina and, if so, what the effect was; also, whether the central nervous system of these iodo-acetated animals was examined histologically.

Before you answer that, I would like to show a slide in confirmation of this last slide of Dr. Noell's which we have been working with; that is the slide on nembutal.

We have been using cats (slide, Fig. 26), using constant conditions, constant intensity and duration of flash. If we take a cat and put it under ether anesthesia, the ERG we receive is that shown at top left. If we allow the ether to wear off, immobilize the animal with succinyl choline and

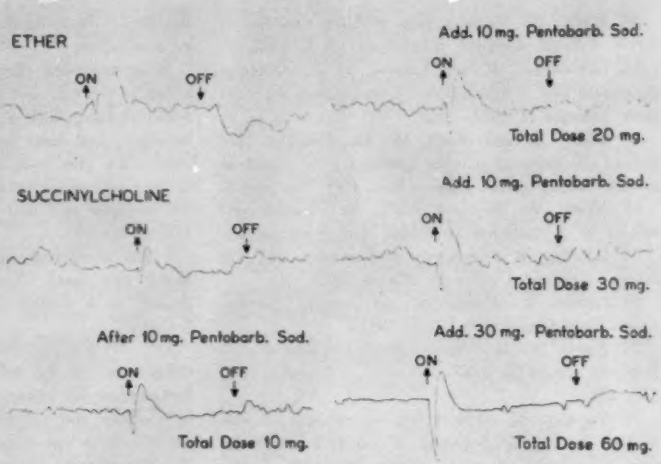

Fig. 26.—Effect of ether anesthesia, succinyl choline akinesia and serial doses of Nembutal upon the ERG showing increase due to Nembutal in increasing doses.

with serially increasing doses of nembutal, we get the changes shown. In succeeding higher doses, as you see, the ERG amplitude increases, while background activity, representing cephalic activity, diminishes."

DR. NOELL: "No; we never tried that. I could not predict what it would do. Generally, we have not been very successful in getting drugs into the retina from the vitreal side. Electroencephalographic effects are not produced by iodoacetate in the rabbit unless administered in overdose causing cardiovascular failure. As to Nembutal effects, your records are just the same as ours in the rabbit. Low doses of Nembutal increase the scotopic b-wave; they also enhance the a-wave; higher doses eliminate the b-wave in a selective manner and unmask the a-wave. Incidentally, the effect of Nembutal on the ERG is faster than its effect on the EEG, and the ERG recovers from selective b-wave depression while the brain effect is still progressing."

DR. CHARLES HAIG, New York Medical College: "I was wondering, Dr. Noell, to what extent your conclusions would apply to the cones. This is all rods you are speaking of, isn't it?"

DR. NOELL: "Yes; that is right; our work mainly relates to rods. Cone cells differ in many respects from rod cells; their metabolic organization appears to be different; they resist cytocidal effect; their synaptic ending has a different structure; the connection between inner limb and nuclear region differs and so on."

DR. DISCHE: "I wonder whether in interpreting these interesting correlations between the metabolism of various sections of the retina and their function one should not also take into consideration the fact these different parts of the retina may differ very strongly in their liability to injury, because what you investigate is dissected retina. If the visual cells (as is most probable and, in fact, is strongly supported by your findings) are much more liable to injury in any form than other parts of the retina, we would, of course, expect significant differences in their respective forms of metabolism. These, however, would be secondary differences due to injury. Differences in the metabolism you observed are in fact those usually associated with injury.

The most surprising feature, I believe, in this picture is the very high consideration of glucose-6-phosphate in certain parts of the retina. This is something which could not be expected, but if it is a well-established fact it really will give some opportunity for thought. On the other hand, I would be somewhat skeptical in interpreting findings concerning individual enzymes of a whole enzyme system as indicative of particular functions.

For instance, I think that malic acid dehydrogenase is a very bad representative of the tricarboxylic acid cycle because, in systems which notoriously lack this cycle, it sometimes is the only enzyme which is present in abundance. So it is certainly not a representative enzyme, for the whole cycle.

Nor can the glucose-6-phosphate dehydrogenase be regarded as very representative of the shunt mechanism, because the shunt mechanism is a very complicated system. One possible function of this enzyme may be providing sufficient amounts of reduced TPN for some specific purposes which have nothing to do with the shunt mechanism as a whole."

DR. NOELL: "We know, of course, that we do not work *in-vitro* with a retina in its natural surrounding and we do not claim that we work with a normal one. I am positive, for instance, that the *in-vitro* respiration values are higher than we can assume to exist *in-vivo*. We hope, but cannot prove

it, that the retinas at different stages of development are affected to a comparable degree by the experimental procedure; we have no evidence to the contrary. Let me say that we search *in-vitro* for a hint on the possible causes of the *in-vivo* effects; the one I gave is a very strong one viewed together with all other evidence. As I specifically said I referred to only a few measurements of Doctor Lowry merely to illustrate the differences in the metabolic properties of the retinal layers. Actually several enzymes of each pathway or subpathway were measured. I may add that the distribution of malic dehydrogenase through the retinal layers is almost exactly the same as that of fumarase. It was not implied that these values are representative of the activities of the systems of which the measured enzymes are a part. Quite differently, these enzymes concentrations are indicative of the capacity of the pathway which contains the enzymes."

DR. M. ALPERN, University of Michigan: "I was intrigued by the discussion of the microhistology of the synapse of the visual cells and the bipolar cells, particularly your description of the vesicles which 'dance before the membrane of the synapse.' I would like to ask if you have attempted to explain the data of P. O. Therman (Acta Soc. Scien. Fenn. N. S. B. 2, 1938, 1-71) who showed, among other things, on the isolated frog bulb that eserine produced a general and symmetrical decrease of all the ERG components while strychnine had an early excitatory effect followed by a paralysis of the ERG. Therman found that acetylcholine also produced a general decrease of excitability of the retinal action potential."

DR. NOELL: "We tried very hard to get retinal effects from strychnine, eserine and quite a number of related drugs. We did not expect effects upon the electroretinogram but were looking for effects upon the synapse between bipolar cell and ganglion cell where acetylcholine may be the transmitter. Since we obtained no effect at this synapse we can only conclude that the poison probably did not reach it. However, there is no reason to assume that acetylcholine might be the transmitter between visual cell and bipolar, certainly not in the rabbit on the evidence of the literature."

CHAIRMAN CRESCITELLI: "I should like to express my personal thanks to Dr. Oliver Lowry of Washington University who generously, and on his own initiative, made unpublished data available to Dr. Noell, data which Dr. Noell has included in his presentation. Dr. Lowry was invited to this symposium but the responsibilities of other commitments prevented him from attending."

Inhibitory Interaction in the *Limulus* Eye—Abstract

H. K. HARTLINE, New York

In the eyes of higher animals the retina is more than a mosaic of independent photoreceptor elements. Even in a primitive eye such as the compound eye of the horse-shoe crab, *Limulus*, neural interconnections exist and interaction takes place among the receptor units (the ommatidia).

The structure and function of the ommatidia in *Limulus* has been described by Hartline, Wagner and MacNichol (1952) and recent studies of its microstructure by electron microscopy have been made by Miller (1957). Each ommatidium is a functional unit. In response to illumination of its facet it generates trains of nerve impulses in the major optic nerve fiber that arises from one of the cells within it. A network of nerve fibers lies back of the ommatidia, interconnecting them. This plexus has been figured in a recent paper (Hartline, Wagner and Ratliff, 1956), and has been shown to mediate the interacting influences.

In the *Limulus* eye the interaction of the receptor units is purely inhibitory. For any ommatidium in the eye the discharge of nerve impulses in the optic nerve fiber coming from it is diminished (frequency lowered) by illumination of other ommatidia in its neighborhood. The amount of inhibition exerted on any given ommatidium (decrease in frequency) is greater the higher the intensity of the illumination on the neighboring ommatidia, the larger the number of them illuminated, and the closer they are to the given ommatidium (Hartline, Wagner and Ratliff, location sited).

Receptors in the *Limulus* eye inhibit one another mutually. If a discharge of impulses is recorded in two optic nerve fibers from ommatidia that lie close to one another in the eye, then, when both receptor units are illuminated steadily, the steady frequency of impulses in each fiber is lower than is the frequency in each when its ommatidium alone is illuminated at the same intensity. The amount by which the steady frequency of discharge of each receptor unit is lowered depends on the degree of concurrent activity in the other and is indeed a linear function of the frequency of its discharge. Thus the response of a given receptor unit is determined by the excitation furnished by the stimulating light shining on it diminished by the inhibitory influence from its neighbor, which, in turn, is the resultant of the excitation of its own stimulus and the inhibition from the first. This mutual interdependence of any two neighboring receptor units in the eye may be described by a pair of simultaneous equations linear in the frequencies of the discharges (Hartline and Ratliff, 1957).

When more than two receptor units are illuminated simultaneously, mutual interaction of all of them affects the response of each. The net inhibition exerted on any one receptor unit is given by the sum of the inhibitory influences exerted on that unit by all of the other active ommatidia in its neighborhood. Two receptors, or small groups of receptors, produce a decrement in frequency of a receptor in their neighborhood (which may be regarded as a "test" receptor) that is equal to the sum of the decrements produced by each acting alone, providing the two groups are sufficiently widely separated that they do not interact with each other (for example, 5 mm. separation, test receptor between them). If the two groups are close enough together to interact their effect when acting together is, as a rule, less than the sum of their separate

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effects, since each group inhibits the activity of the other and hence reduces its inhibitory influence. However, the test receptor or a small group of ommatidia in its neighborhood that may be illuminated with it may interact with the two groups and affect the net inhibitory action. For example, disinhibition can be demonstrated under certain conditions. Indeed, a variety of quantitative effects has been observed for different configurations of three such groups of receptors. These effects may be explained by extending the system of simultaneous equations so that they apply to many interacting elements. Applied to three interacting receptors or receptor groups, equations have been derived that account quantitatively for the variety of effects observed in the various experimental configurations of retinal illumination that have been used (Ratliff and Hartline, in press).

Inhibitory interaction in the retina results in the enhancement of brightness contrast. Since the inhibition is greatest between elements that are close together, contrast is greatest at borders and edges in the retinal image, where steep gradients of intensity exist. Direct experimental evidence for this has been obtained from the eye of *Limulus*.

The inhibitory interaction distorts the pattern of neural activity in the optic pathway, so that the pattern becomes more than a mere reproduction of the intensity distribution on the retinal mosaic. At the sacrifice of less important fidelity, certain significant features of the distribution of light and shade are accentuated. Inhibitory interaction is an integrative action that takes place at a very early stage in the visual process.

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DISCUSSION

DR. JACOBSON: "I would like to talk about inhibition in a mammal for a few minutes, if I may.

Dr. Noell and I, as we discussed before, both noticed the effect of Nembutal on the ERG; and we in our laboratory, Mr. Gestring and I, began to wonder why this seeming increase in the amplitude of the ERG occurred when we gave a cat Nembutal.

This slide (Figure) shows, on the left, the ERG of each of a cat's two eyes, as recorded with cotton wick electrodes while the cat was under succinyl choline akinesia. The skull was open, the optic nerves exposed and a string had been passed around one nerve just distal to the chiasm.

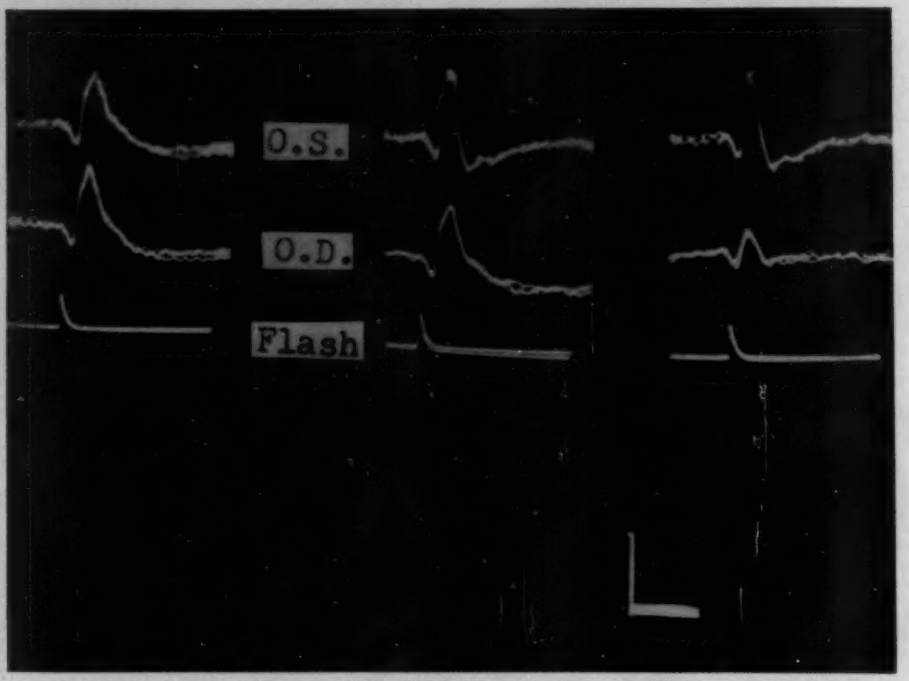
The center two responses show the effect of pulling up on the string about the optic nerve, thereby sectioning the nerve of the eye at top. There is an increase in the amplitude of the response.

The right hand two records show the effect of hexamethonium, a ganglionic blocking agent. Following intravenous hexamethonium, in the eye with an optic nerve, the amplitude of response is immediately and markedly diminished. This does not occur in the eye whose nerve is cut.

This hexamethonium effect is very constant, and is not due to blood pressure drop, since we can not reproduce it by blood loss, such as cutting the carotids, or with other drugs which cause a greater drop in blood pressure. The drop in blood pressure with hexamethonium is not great.

One further thing, going back to some of Dr. Noell's work, is that we enucleated some eyes, obtained retinae, put electrodes on the surface of open cat's retinae. We then dropped on the surface of these retinae a number of different kinds of drugs, one of which was nembutal. We found only a slight diminution, which would seem to be evidence that it is not a direct retinal effect.

It is our impression that these findings indicate the existence of a cephalic inhibitory-facilitory center for control of ERG amplitude, and that the efferents of this center are mediated by the optic nerve.



ERG of each eye, (left, top; right, bottom) of a cat under succinyl choline akinesia. Traces in left column control. Center column immediately following left optic nerve section, just distal to the chiasm. Right column following intravenous hexamethonium.

Hexamethonium and Nembutal affect the center in such a manner as to cause reduction and increase in the ERG, respectively, and section of the optic nerve prevents these effects by blocking the efferent pathway."

DR. KENNETH T. BROWN, Wilmer Institute: "Dr. Jacobson's remarks on inhibition in mammals lead into some findings in our laboratory. We have been using micropipette electrodes in the retina of the unopened cat eye and recording from single units of the ganglionic and inner nuclear layers. We have now found an inhibitory type of response which has certain distinctive features not described previously in mammals.

Of course the 'on', 'off', and 'on-off' discharges which have been recorded from mammalian ganglion cells by surface electrodes are well known. Previous investigators have seen inhibition of ganglion cells during the stimulus, but only in association with the 'off' discharge. The new type of response we have found is only inhibition, with no 'off' discharge. When the stimulus is turned on the spontaneous discharge, or the discharge resulting from the background illumination, is inhibited. The strong initial inhibition gradually weakens during the stimulus; this adaptation of inhibition is like that seen in *Limulus*. When the stimulus is turned off, inhibition outlasts the stimulus for a variable period and there is typically a gradual return of the pre-stimulus discharge rate.

The purely inhibitory response has been found from certain cells of both the ganglionic and inner nuclear layers. This response is seen best with a small spot in the center of the receptive field of the cell which is being recorded, since the peripheral portions of these receptive fields contribute 'on' or 'off' discharges. The inhibitory response is graded with stimulus intensity. In some part of the intensity range, however, an 'off' discharge has been found in all units studied to date.

The purely inhibitory response from the cat retina is superficially like the lateral inhibition response in *Limulus*, but details of how the responses are produced differ between the two species. Our tentative interpretation is that the cat can see at least as well as *Limulus*."

DR. M. ALPERN: "I would like to ask about the phenomenon of disinhibition. As far as I know it has never been demonstrated in a vertebrate retina. Do you find it?"

DR. K. BROWN: "We have not done enough work yet on this discharge pattern in relation to

the receptive-field characteristics to say very much about that."

DR. M. ALPERN: "My impression from psychophysical data is that this does not occur, at least Piéron (Journale Psychol. *32*, 1-24, 1935) looked unsuccessfully for it.

It seems to me that this phenomenon may be of importance, not for the vertebrate eye but for the compound eye. In this latter case each ommatidium has its own lens system. If these lenses are similar to lenses as we commonly think about them, then each ommatidium must have an image formed within itself by its own lens system. That is, an image of a fairly large section of the visual field must be formed on each ommatidium. It would not be expected that the images in successive ommatidia would differ very much from one another. It is difficult to understand, if each ommatidium functions as a single unit as Dr. Hartline's experiments so beautifully demonstrate, how compound eyes have any resolution. And yet we know from the work of Hecht and Wolf (J. Gen. Physiol. *12*, 1929, *727*), among others, that the bee for example has remarkably good visual acuity (only one hundredth that of our own).

I do not believe that the directional sensitivity of the ommatidium is sufficiently large to account for these phenomena all by itself. Waterman (Proc. Nat. Acad. Sci. *40*, 1954, *252*) studied this matter in some detail. He nevertheless found that a light could be moved through a visual angle as large as 90° and still produce an electrical response in the same ommatidium. I would like to suggest that the phenomenon of disinhibition described by Hartline offers another way of sharpening up the contrast of objects so that the compound eye achieves a respectable amount of resolution."

DR. H. K. HARTLINE: "The emphasis perhaps should not be put on disinhibition so much as on the inhibition. Disinhibition is a result of the operation of a mechanism in which the inhibitory influences act at the place where the discharge is generated. It is quite conceivable that one could also have inhibition which depends not upon the inhibited level of response but rather upon the level of stimulation of the receptor. This plan would serve equally well for contrast enhancement.

The role of inhibition within sensory systems is eliciting much interest. Bekesy has been interested in this for a long time. You may know that inhibition in the ear was described by Davis and Galambos some time ago; and the auditory physiologists are much interested in the role which it may play in improving the resolution for the patterns of activity on the basilar membrane. Bekesy now is interested in similar effects which he can observe in the skin, where one can get very striking sharpening of the patterns of tactile stimulation.

The *Limulus* eye is an apposition eye. The field of vision of each ommatidium is a few degrees in diameter (considering only the central sensitive portion, and avoiding effects of scattered light). This eye must have extremely poor resolution.

The study of inhibition in this eye may be useful in furnishing a model of an operational principle which perhaps may find application in other parts of the nervous system as well as the visual."

DR. SAMUEL GELFAN, New York Medical College: "Dr. Hartline said nothing about the mechanism of the inhibition which he described; I am not sure whether he wishes to say anything about it.

Inhibition in the vertebrate motoneuron, according to the Eccles school, is associated with an hyperpolarization. According to Kuffler, inhibiting impulses (at least in the nerve synapsing upon the stretch receptor cell of the crayfish) drive the membrane potential of the affected neuron in the direction of repolarization, or toward its equilibrium level. At any rate, synaptic transmission in these cases is between axon and soma or axon and dentrite. In the *Limulus* eye, however, inhibitory transmission appears to take place between the branches of different eccentric cell axons. That is, the synapse is an axon-axon one. Do we have here, in the case of the *Limulus* optic nerve, a similar mechanism of inhibition but transmitted from axon to axon? In the cat motoneuron inhibition is blocked by strychnine or tetanus toxin. If a similar mechanism operates in the *Limulus* eye, disinhibition might be achieved by strychnine."

DR. H. K. HARTLINE: "I think Dr. MacNichol might talk about hyperpolarization, if he will."

DR. MACNICHOL, Johns Hopkins: "I have been unable to demonstrate inhibitory hyperpolarization in a normally functioning eccentric cell, in the *Limulus* ommatidium, though I have tried very hard.

When we recorded from a micropipette inserted into an eccentric cell there was a slow, positive potential change (depolarization) in response to light. In the body of the eccentric cell this was one or two millivolts in amplitude. Superimposed on this slow potential were large, positive spikes 20 to 40 m. v. in amplitude.

When surrounding ommatidia were illuminated the spike discharge would slow down or was completely inhibited, depending upon the strength of the illumination. However, instead of a negative slow potential (hyperpolarization) when the inhibitory light was turned on, the microelectrode became still more positive (further depolarization). In only one experiment was there ever any evidence of hyperpolarization. In this case the punctured eccentric cell was firing spontaneously and had lost all response to light. Illumination of neigh-

boring ommatidia caused a decrease in spike frequency and a negative going, slow potential. Since it was impossible to prevent scattered light from reaching the ommatidium from which a recording was being made, it is possible that the depolarization usually produced in the eccentric cell when neighboring elements were illuminated was due to scattered light. This depolarization may have masked the inhibitory hyperpolarization as measured in the body of the eccentric cell. The inhibitory component was revealed in the experiment in which the eccentric cell did not respond to illumination because scattered light could have no effect."

DR. STEPHEN W. KUFFLER, Johns Hopkins University: "First of all I would like to give the *Limulus* some support from the vertebrate retina. The inhibitory surround in receptive fields of cats also supports the idea that this mechanism exists for the production of contrast. Furthermore, if you put the eye in darkness, the inhibitory surround drops out; in other words, your receptive field becomes uniform when one suspects that contrast also disappears.

In respect to mechanism I would like to expect that the processes in *Limulus* will turn out to be similar to those in the spinal motoneurones and in stretch receptor cells. After all, most organisms are designed in the image of Hartline's *Limulus* eye."

DR. MACNICHOL: "We have some evidence to support Dr. Kuffler's suggestion that the site of inhibition is indeed some distance away from the body of the eccentric cell. In fact we think it is in its axon. In the first place the eccentric cell does not appear to discharge an action potential. Spikes recorded externally from this cell were always positive whereas they would be expected to be negative when the electrode was outside; changing abruptly to positive when the electrode penetrated the cell. Secondly, the spike potential was never as large as the resting potential indicating that it was conducted electrotonically from some distance. Thirdly, Dr. Hartline has shown that there is a region along the neck of the ommatidium in which the spikes reverse sign abruptly which indicates that they originate in the axon and are blocked before reaching the eccentric cell. Tomita (J. Neurophysiol. *20*, 245-254, 1957) has some experiments that furnish additional evidence on this point.

From the foregoing discussion it is evident that the site of origin of impulses is in the axon near the eccentric cell. The presumably inhibitory synapses shown in Dr. Miller's sections are also located in this region. The body of the eccentric cell is therefore nearer to the site of origin of the excitatory potential and further from the site of origin of the inhibitory potential than the place where spike activity originates. Hence, we may except the excitatory potential to predominate in recordings made from the body of the eccentric cell although the inhibitory effect is probably proportionately much stronger where the impulses originate."

The Human Electroretinogram

LORRIN A. RIGGS, Providence, R. I.

Previous speakers have shown the progress in understanding visual problems that has been achieved by experimentation on animals. By comparison, the human being is obviously a poor subject for physiological studies because of the limitation that we may not inflict damage or even serious discomfort upon the person. Thus we must forever remain on the outside, trying to look in. We may recall, however, that the human electrocardiogram has been useful despite the necessity for an external location of the recording electrodes. Progress has likewise been made in the 28 years of research on the human electroencephalogram. It turns out that the eye, like the heart and the brain, can be made to yield significant information about its activity through the use of electrical recording techniques. It is the purpose of the present paper to assess the progress that has been achieved to date on the human electroretinogram or ERG.

Development of Recording Techniques

It was eighty years ago that Dewar [1] first succeeded in detecting an electrical response in a human eye. To do this he had the subject lie down and open his eyes wide. Then a ring of paraffin was brought to rest on the sclera in such a way as to encircle the cornea. The inside of the ring was filled with a dilute salt solution. This covered the cornea and served as an electrolyte between it and a metallic electrode. The reference electrode was applied to the hand of the subject, and the two electrodes were then connected to a sensitive Thomson galvanometer. Dewar [1] describes the experiment as follows:

Brown University.

This report was prepared under a contract between Brown University and the Office of Naval Research.

"By a laborious procedure of education it is possible to diminish largely the electrical variation due to the involuntary movements of the eyeball, and by fixing the eye on one point with concentrated attention, another observer, watching the galvanometer, and altering the intensity of the light, can detect an electrical variation similar to what is seen in other animals. This method, however, is too exhausting and uncertain to permit of quantitative observations being made."

In 1925 Hartline [2] described experiments in which he used moist thread electrodes and saline-filled goggles to make contact with the eyes. The resulting string-galvanometer records reveal the negative *a* and positive *b* and *c* waves, features of the human ERG that have since been studied in more detail with electronic methods of recording. Of greatest importance was Hartline's demonstration that similar results could be obtained in animals, with excised eyes and with the intact preparations. This gave the necessary corroboration for Dewar's conclusion that the retina was responsible for the results even when a remote reference electrode was used in connection with an electrode on the cornea of the intact eye.

Sachs,[3] in 1929, showed that the human ERG was typically dependent on the night (scotopic) visual system of the retina. He also reported, however, that the protanopic form of color blindness resulted in a diminished ERG in response to red light. In 1933 the first electronically amplified human ERG was recorded by Cooper, Creed and Granit [4] and at about that time Granit [5] analysed the ERG into separate component reactions, one of which (PIII) was negative and the other two (PII and PI) with dif-

Fig. 1.—Contact lens electrode and forehead electrode for recording the ERG.

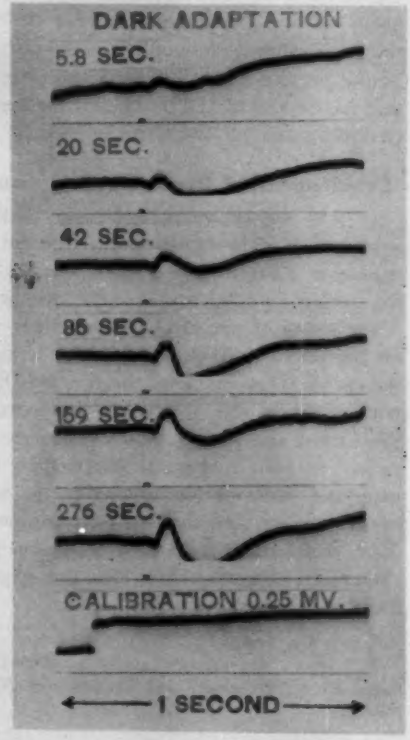


Fig. 2.—Records obtained in 1941 with the first contact lens electrode. The black dot below each record signals the moment of the test flash. This produces a small negative *a*-wave followed by a positive *b*-wave and a much slower positive *c*-wave. The ERG increases progressively during dark adaptation.

ferent time relations, were positive in polarity.

In 1940 Bernhard [6] employed flickering light to distinguish clearly the photopic from the scotopic components of the human ERG. The Bernhard experiments made use of a recently developed dual beam cathode ray tube and push-pull amplification. In these studies the connection with the eye was made by the use of a thread electrode moistened with saline solution.

Modern work on the human ERG has made use of plastic contact lenses to establish a stable electrical connection with the cornea. This method, first described by the author [7] in 1941 did not come into general use until after World War II. The contact lens electrode has (Figure 1) minimized the variation in results due to eye movements and reflex winks. Experimental sessions of two hours or more have become possible, thus permitting the quantitative study of dark adaptation, spectral sensitivity and other visual functions. Figure 2 shows some early records taken during dark adaptation. The clinical application of the ERG has also been based on the use of contact lens electrodes, several forms of which have been developed specifically for use with patients.

Having sketched the development of apparatus for recording the human ERG, I should like now to discuss some of the major results that have been achieved and the problems that remain to be solved.

Origin of the Human ERG

Of primary interest is the question of the site of origin of the ERG. Obviously this is not a question that can best be answered by work on the intact human eye and it will be discussed only briefly here. The animal experiments of Dewar and McKendrick,[8] Kühne and Steiner,[9] Hartline [2] and others have clearly shown that the excised eye yields an ERG similar in wave form to that of the intact eye. Thus it may be concluded that even the remotely located electrodes used with human subjects are not significantly affected by optic nerve impulses or other extra-ocular activity. Furthermore, a typical ERG has often been elicited from the isolated retina of a frog or other cold-blooded animal, showing that the responses originate primarily within the retina itself. Finally, certain considerations strongly suggest that the ERG arises chiefly from the receptor cells or bipolars or both. A brief summary of these considerations is as follows.

First, the ERG manifests itself maximally when the two recording electrodes are located on opposite sides of the retina. The histology of the retina is such that the receptor cells and the bipolars are the chief structures occurring in sufficient numbers and suitably oriented to constitute a battery for generating the ERG. Second, Granit [5] and others have shown that the physiological inactivation of the ganglion cells has no effect on the ERG. Third, clinical studies by Karpe [10] and others have shown that despite an impairment of the ganglion cells and optic nerve there is sometimes a survival of the ERG. Fourth, Noell [11,12,13,14] has studied the ERG in an extensive series of studies of retinal impairment produced by iodoacetate, sodium azide, and sodium iodate. His conclusion is that the ERG arises principally in the inner portions of the receptors and the outer region of the bipolars. The negative (*a* and *d*) waves may originate more within the receptors and the positive (*b*) component in the outer region of the bipolars. Finally, several recent studies with microelectrodes have localized the major ERG effect to approximately the same retinal level. Brindley [15,16] and Svaetichin [17, 18,19,20] have implicated the receptor cells alone, but Tomita [21,22] has favored the bipolars as the major source of the ERG. We may confidently expect that the next few years will bring combined histological and microelectrode data that will localize even more exactly the various components. It appears likely that the components are differently localized and that important interaction effects are occurring both horizontally and vertically within the retina. We may

reasonably infer that the human ERG reflects the response to light of large numbers of rods and cones, probably modified by the activity of other structures located primarily in the bipolar layer of the retina.*

Scotopic Components of the ERG

The duplex nature of human vision is nowhere more clearly shown than in the electrical reponses of the retina. The rods, more than 120,000,000 of them in each eye, tend to dominate the cones, which number scarcely 7,000,000. Furthermore, the rods are more widely dispersed throughout the retina while the cones are more heavily concentrated in the small central region of the macula. The result is that large-sized ERG's can only be recorded when the eye is dark-adapted and the stimulus light pervades a large portion of the retina. Indeed almost all of the human ERG's recorded up until fifteen years ago were primarily of scotopic origin. The photopic components were generally too small to be registered by apparatus then available and the relatively large scotopic responses overshadowed them, especially when the eye was in a dark-adapted state.

A part of the difficulty with early human experiments on the ERG was the failure to recognize the significance of stray light. Fry and Bartley [23] had shown in 1935 that the ERG of the rabbit was often elicited mainly by light scattered from a focal area to peripheral regions of the retina. This point was finally made clear in the human ERG when Boynton and Riggs [24] and Asher [25] reported that light directed to the blind spot was nearly as effective in arousing the ERG as a similar light falling on the fovea or periphery. Since there are no receptors in the blind spot, this demonstrates the effectiveness of scattered light. The recent study by Crampton [26] of the effects of a glare source confirms the fact that the ERG arises primarily from areas not directly illuminated by a stimulus field of moderate size.

The size of the ERG is hard to reconcile with the subjective brightness of the stimulating light unless the factor of scatter is considered. Blue light, for example, scatters much more effectively than red. Hence a small blue patch of light is found by Riggs and Johnson [27] to arouse a disproportionately large ERG when compared to a small red patch of equal subjective intensity. Only the use of very large stimulating fields of low brightness can serve to reduce this excess blue responsiveness to a point that the ERG-determined curves resemble the psychophysical ones for spectral luminosity, at scotopic (Boynton [28]) and photopic (Armington [29]) levels.

The early quantitative study of human dark adaptation by Johnson [30] was concerned with the scotopic components of the ERG. Likewise the clinical application of the ERG developed by Karpe in 1945 was largely in relation to the scotopic system. Karpe [10] found drastic reductions in ERG in cases of widespread peripheral impairment such as retinitis pigmentosa, siderosis, vascular disease and congenital night blindness. It is thus clear that most of the results that Karpe obtained at that time were due to the responses of the rod receptor system. In our own laboratory we [31] have obtained ERG's from a number of night blind subjects. An ERG from a congenital night blind man is shown above, for example, in Figure 3. Below is an ERG elicited from a normal eye by a much weaker stimulus. The dark adaptation curve for this subject, measured by Dr. Louise Sloan is shown in Figure 4. Measurements of his ERG are shown in Figure 5 and these reveal similarly the impairment of his night vision as compared to responses from a normal eye.

* It is an interesting fact that the ERG can be recorded so well by electrodes rather remote from the retina. It is true that the receptor side of the retina is in intimate contact with the vascular system in the choroid, and that the anterior side of the retina is in turn connected with the vitreous, which is likewise a relatively good conductor. It may therefore be that the cornea, relatively well insulated from the remainder of the body, exhibits the potential changes developed across the retina and communicated to it through the vitreous and other internal media of the eye.

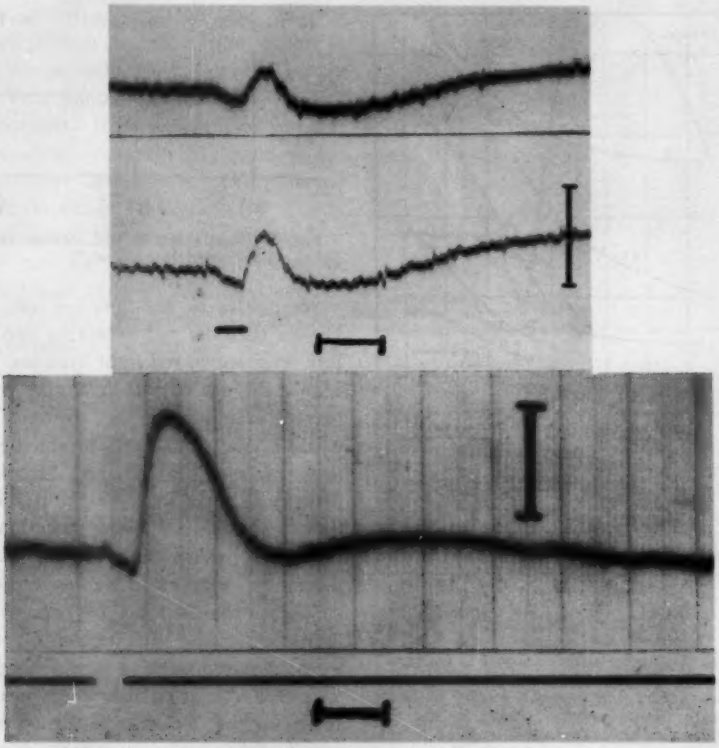


Fig. 3.—Night blind and normal ERG records after dark adaptation. The first two ERG's are from congenitally night blind Subject G for test flashes of 10^6 and 2×10^6 footlamberts respectively. The third ERG is from a normal subject with a test flash of 2×10^4 footlamberts. The short vertical line denotes 500 microvolts. The short horizontal line denotes 0.1 sec.

As late as 1947 Granit [32] stated that, for the human ERG, "At high intensities a small *a*-wave often develops, but there is never any off-effect. The appearance of an off-effect should always be suspected as an artifact. . . . The shape of the electroretinogram is the same whether it is taken from the central or the peripheral retina. . . ." In 1949 Riggs, Berry and Wayner [33] reported an extensive series of determinations of spectral sensitivity, using the magnitude of the *b*-wave of the human ERG as the response indicator. The conclusion was again drawn that this *b*-wave resulted chiefly from the scotopic visual system, even with moderate degrees of light adaptation. It was evident, however, that orange and red light produced relatively larger responses in the light-adapted than in the dark-adapted eye. While no Purkinje shift of wavelength maximum was found at that time, the differential effect of red light foreshadowed the later experiments that have indeed demon-

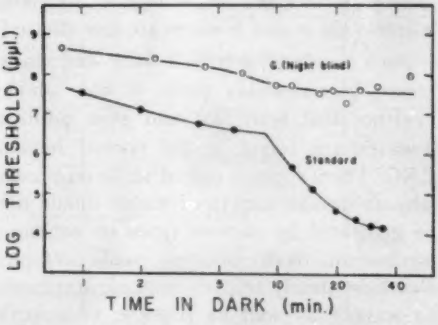


Fig. 4.—Adaptometer data obtained on Subject G (night blind) and on standard observers by Dr. Louise Sloan.

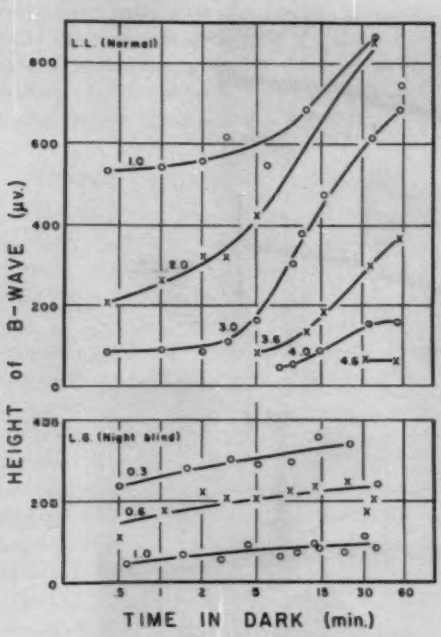


Fig. 5.—Height of the *b*-wave of the ERG as influenced by dark adaptation. Subject L (normal) above, Subject G (night blind) below. The number beside each curve denotes the density of filter, in logarithmic units, used to attentuate the test flash from its original value of 2×10^6 footlamberts.

strated the shift from scotopic to photopic responses at high intensity.

Photopic Components of the ERG

It is obvious that the early restriction of the ERG to scotopic visual processes was a serious handicap for both clinical and experimental work. Human vision is dependent on the photopic system for all the finer discriminations of color, form and motion and the fovea is of course particularly well represented in the visual projection areas of the cortex. Since the fovea contains but a small proportion of the receptors and bipolars of the retina it becomes important to seek out ways by which to uncover the photopic responses that have been so difficult to measure in the human ERG. We may now summarize some of the means that have been employed to accomplish this.

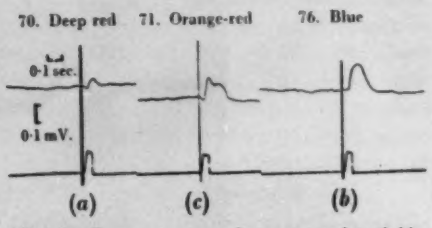


Fig. 6.—Responses to red, orange-red and blue lights as recorded by Adrian.[35]

Responses to red light.—First, it is well known that the rod receptors are relatively less sensitive to red light than are the cones. Hence the use of red light may well be expected to minimize the scotopic responses while at the same time producing photopic ones of measurable size. Motokawa and Mita[34] in 1942 used bright flashes of red light and obtained brief spike-like positive responses that they called "*x*-waves." Adrian[35] independently investigated responses of this kind to orange and red lights and showed (Figure 6) that they were photopic positive waves having faster time characteristics than the typical *b*-waves occurring in response to lights of shorter wavelengths. Later Armington[36] and Schubert and Bornschein[37] were able to show that these *x*-waves were absent or greatly diminished in the case of red light flashes delivered to protanopic (red-blind) human subjects. Schubert and Bornschein[38] also noted a diminution of the ERG with hypoxia, and the recent report of Brown, Hill and Burke[39] indicates that the *x*-wave is greatly diminished in moderate hypoxia, whereas the *a*- and *b*-waves are less affected.

As a result of Adrian's work and many recent investigations there is now ample evidence that both fast and slow positive *a*-waves are found in the normal human ERG. There appears indeed to be no reason why an infinite variety of waves should not be generated by various types of response mechanism, both photopic and scotopic. Red light clearly arouses photopic responses (*x*-waves) as well as scotopic (*b*-waves) so that two distinct positive waves appear on the ERG. This is more or less true for

yellow and green as well; but the distinctness is not present for blue light, where a relatively simple and slow *b*-wave usually appears. This finding is not clearly understood at present. It may simply mean that the responses of blue-sensitive cone receptors are so weak that they are overpowered by the rod responses. Alternatively, it may mean that cone receptors specialized for blue light are as slow in their response characteristics as are the rods. Still another possibility, favored by some, is that there are no blue-sensitive cones and blue vision is mediated by the rods.

Light adaptation.—A second scheme for emphasizing photopic responses, as compared to scotopic, is simply the use of a light-adapted eye. Light adaptation greatly reduces the sensitivity of the rod receptors without so much affecting the cones. Adrian [35] has clearly exhibited this effect in comparing responses to various wavelengths of light in different stages of adaptation, as shown in Figure 7. Also on the basis of comparing responses to red and blue stimuli in light and dark adaptation, Armington, Johnson and Riggs [40] were able to sketch the probable contributions of the photopic and scotopic responses to the recorded human ERG as shown in Figure 8. It is seen that both contributions are diphasic, so that both the *a*- and *b*-waves of the normal ERG are seen to be composed of photopic and scotopic components.

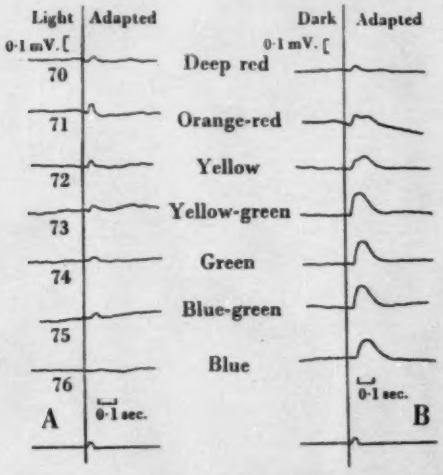


Fig. 7.—The human ERG in response to various wavelengths of light during (A) light adaptation and (B) dark adaptation, as recorded by Adrian.[35]

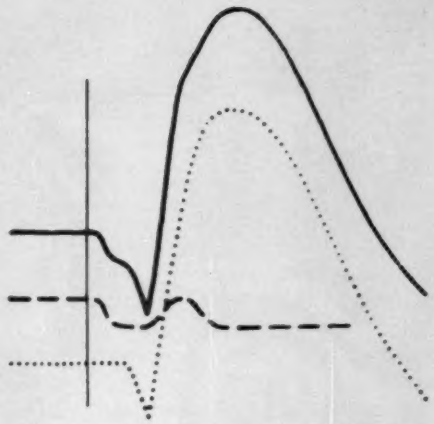


Fig. 8.—Analysis of the ERG in dark adaptation (solid line) as the resultant of photopic (dashed line) and scotopic (dotted line) components as derived by Armington, Johnson and Riggs.[40] Note the similarity of the photopic component to responses shown in Fig. 2 for a relatively light adapted eye, in Fig. 3 for a night-blind eye and in Figs. 6 and 7 for a deep red stimulus.

Selective adaptation to lights of various wavelengths has been employed by Armington and Biersdorf [41] with some evidence for specific wavelength effects. Recently, Biersdorf and Armington [42] have studied the ERG resulting from a sudden shift of wavelength from an adapting field to a test stimulus. They have thus obtained evidence for peaks of spectral sensitivity at 500 mμ. and at 620 mμ.

Flashes of short duration.—A third method of emphasizing photopic responses is to use stimulus flashes of short duration. The recent studies of Johnson and Bartlett [43] and Alpern and Faris [44] have shown that the critical duration for photopic conditions is shorter than that for scotopic, so that a maximal photopic response can be achieved with durations well below those that yield maximal photopic ones.

Off-effects.—Fourth, it is possible to study the off-effect rather than the on-

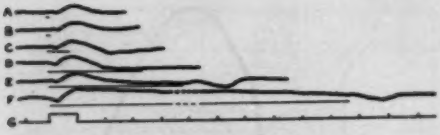
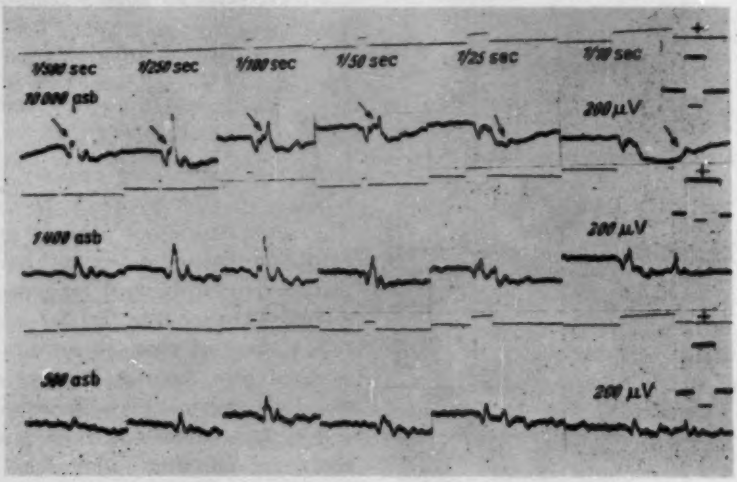


Fig. 9.—Tracings of the ERG in response to white lights of varying duration. Records A to F are for durations of .010, .015, .075, .33, .48 and 2.45 sec. respectively. Time in tenth seconds and a calibration of 100μv. are shown in G.

effect of light. Figure 9 illustrates some early records of exposure duration, made by the author with the contact lens electrode in 1941. These show clearly the development of an off-effect after prolonged exposure to light. Dodt [45] and Best and Bohnen [46] have studied the off-effect in the light-adapted eye. Figure 10 shows that the off-effect may appear even with brief exposures. Multiple spike-like waves appear in the off-effect, suggesting the possibility of numerous inhibitory and excitatory events, possibly the events underlying the subjective experience of after-images. Care must be taken, however, to eliminate pupillary responses and other artifacts in this situation.

Responses to flicker.—Finally, it is possible to combine the features of short exposure, off-effects and light adaptation by the use of prolonged stimulation with flickering light. Flicker has been a favorite stimulus pattern for the human ERG as it has in psychophysical studies. Figure 11 shows responses obtained by Adrian [35] in which repeated flashes of red light gave relatively constant results while with blue light the responses diminished with each succeeding flash. More recent flicker studies have included those of Dodt,[47] Bornschein and Schubert,[48] Armington and Biersdorf,[41] Johnson and Cornsweet,[49] Burian,[50] Best and Bohnen,[51] Goodman and Iser,[52] Vanvysek,[53] Heck [54] and others on the normal human eye. In these studies it has clearly been established that flicker responses can be obtained over the range usually covered with psychophysical experiments. Slow rates of flicker (typically below 20 cycles per second) enable the rods as well as the cones to take a prominent part in the ERG. Higher rates seem to yield less activity from the rods and thus to bring out cone activity. Responses to flicker are seldom simple, sinusoidal waves. Often the positive and negative components of succeeding flashes impede or reinforce one another in such a way as to produce sharp, spiky components in a given frequency range.

Fig. 10.—Records obtained by Best and Bohnen.[46] The off effect on the human ERG is designated by the arrow for the responses at the indicated exposure durations for the highest intensity (10,000 asb) of the stimulating flash.



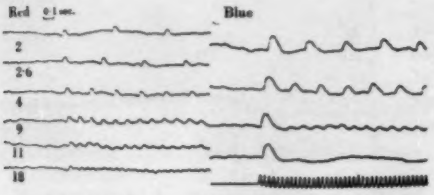


Fig. 11.—Records obtained by Adrian[35] in response to repeated flashes of red and blue lights. Flash frequency is designated by the number opposite each record. The scotopic ERG for blue light quickly fails due to light adaptation, so that little activity is seen with prolonged exposure to high rates of flicker. The photopic ERG is diminished relatively little in this way, as is evident in the responses to red.

Already the flicker method has yielded spectral sensitivity functions closely approximating those of psychophysical ones, provided that sufficiently large stimulus fields are employed, as Armington[29] has shown. The flicker method is also a convenient one for clinical use. Dodt and Wadensten,[55] and Iser and Goodman,[56] for example, have emphasized the necessity for flicker records along with single-flash records for a proper assessment of retinal function.

Recently Henkes et al.[57] have made use of selective amplification to accentuate the responses to flickering light. This applies a principle first suggested by Granit,[58] namely the use of a frequency analyzer in the output of the amplified ERG. In the records of Figure 12 the analyzer is tuned to the particular frequency of the flickering light, whereupon it responds to energies too small to be noticeable on the typical ERG record. Henkes employed this method in a series of recent experiments in our laboratory. Figure 13 shows a typical record in which photopic responses were emphasized by adaptation to blue light. An alternative scheme that we have recently employed in our laboratory is to sweep the frequency analyzer through a small range of frequencies, from slightly above to slightly below the frequency of the stimulating light. Figure 14 shows that this permits an easy comparison between energy at the maximum and energy to either side, thus ensuring that the response to the flickering light *per se* has been measured.

In conclusion, the human ERG has come of age to the extent that it can now be used to measure photopic as well as scotopic activity within the eye. It is, however, a mass response and cannot yet be used for any such purpose as perimetry or measurement of visual acuity. Its clinical and scientific value may well lie in the fact that it indicates primarily the activity at the receptor and bipolar levels of the retina. Its results can thus be compared with psychophysical judgments of phenomena that are dependent on the entire visual system.

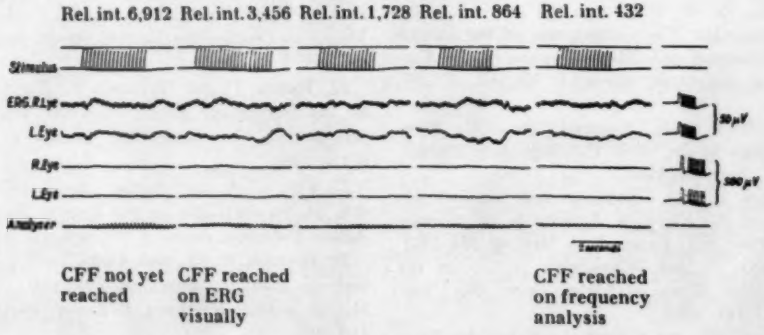


Fig. 12.—The principle of selective amplification as used by Henkes et al.[57] with the human ERG. The typical ERG record, even at high gain, fails to reveal responses that are still clearly evident in the output of the tuned frequency analyzer at a flash frequency of 6.3 per sec.

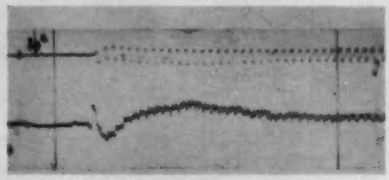
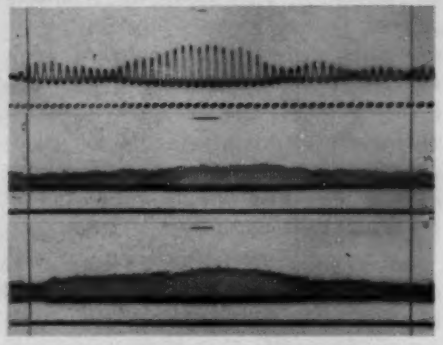


Fig. 13.—Responses obtained by Henkes with the tuned frequency analyzer (upper record) and DC amplifier (lower trace) for flickering white light superimposed on a steady surrounding field of blue light. The onset of the flickering light is followed by a large scotopic single response wave followed by mixed photopic and scotopic responses to the individual flashes at 12 per second.

Fig. 14.—Frequency analyzer output as flicker rate is gradually reduced through the rate to which the analyzer is tuned (movement designated by short horizontal signal). The three records display responses of the analyzer tuned to flash frequencies of 10, 25 and 40 per sec. respectively.



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DISCUSSION

DR. H. RICHARD BLACKWELL (University of Michigan): "Dr. Riggs, you remarked that the photopic response was better recorded with a large field of flickering light than with a small field. Since the cones are presumably concentrated in a small foveal area, isn't this a surprising result?"

DR. RIGGS: "Yes; this is a paradoxical situation indeed. It goes back to the fact that scattered light, stray light, is our big difficulty. If you use

a small field, most of the effect of the light is on the peripheral regions of the eye. So, instead of accomplishing what you want (namely, to concentrate it on the photopic cones), you are in fact throwing the light everywhere you do not want it. So it becomes necessary, therefore, to use a fairly large field, and to knock out the rods not by location, but by a procedure of adapting them to the flickering light, and using rates of flicker which are high enough to discourage the rods and bring in the cones.

DR. BLACKWELL: "Wouldn't the use of red light also be helpful in isolating the photopic response?"

DR. RIGGS: "Red flickering light; yes. This would be very advantageous. In fact, this has been used in several studies now, where Dr. Jacobson, for example, is working with this to get to the macula of the eye, as well as possible further studies of macular degeneration. So the use of red light does help out in the use of flicker."

DR. HAIG: "I am a little confused on that issue of stray light. The center of stimulation will always be very much brighter than the scattered light, won't it?"

DR. RIGGS: "Yes, indeed, it is. I think the explanation probably is in the mass character of the response; that is, if you have a relatively few cells which are active, you just do not pick it up at the cornea. You could, if you were down in there, as we have done with the frog retina, for example. We can get similar potentials by putting a microelectrode down where the event has occurred."

DR. HAIG: "If you do not have any trouble psychophysically with that isolation."

DR. RIGGS: "No; fortunately, it works the other way with the psychophysical. The cortex is well supplied with neural connections from the fovea, so that all the emphasis of the psychophysical is against the rod system as opposed to the ERG, where the rods are favored."

DR. AUSTIN H. RIESEN (University of Chicago): "I would like to ask, in your human b-wave, and what I took to be the b-wave on the rabbit's ERG which Dr. Noell showed us this morning, his b-waves had two, sometimes three different humps, apparently, in the record. I wondered whether there is a species difference here, or what those actually might mean."

DR. RIGGS: "I think not so much a species difference as a difference in experimental conditions, very often; that is, I am sure Dr. Noell also recorded rather simple b-wave responses in the rabbit, under conditions of dark adaptation, for example. When you change the conditions of adaptation and the wave length of the light, and so on, you produce qualitative changes in the wave form. This is true of the rabbit as well as of the human eye.

There are, of course, species differences; but, within each species, you do get quite a diversity of wave form."

DR. KENNETH T. BROWN (Wilmer Institute): "As Dr. Riggs pointed out, one of the greatest problems with the ERG is where the different components come from. Since we have been concentrating on this problem, perhaps I should mention some of our results with micropipette electrodes in the retina of the unopened cat eye.

"The cat ERG consists of four major components. In addition to the a-, b-, and c-waves, which are well known, there is a DC component which can be isolated clearly. Physiological methods have been developed for localizing the electrode in the retina, and these methods have proved much more precise than depth recording from the retinal surface. All four components of the ERG reverse polarity, with respect to vitreous recording, as the electrode passes through the retina. The only reversal point which can be localized precisely is that of the c-wave, which inverts when the electrode penetrates Bruch's membrane. The amplitude of each ERG component has been plotted as a function of retinal depth, and each component has been localized by finding the point in the retina at which the component has maximal amplitude. This method shows the b-wave and DC component to be generated in the external plexiform layer or the inner margin of the outer nuclear layer, while the a-wave and c-wave are generated very close to the inner face of Bruch's membrane. Xylocaine injected into the vitreous humour abolishes the b-wave and also seems to abolish the DC component, while the a-wave and c-wave remain unaffected. Since xylocaine blocks impulses, it should abolish those ERG components which are relatively far back in the retina and dependent upon propagated activity.

"Extracellular recordings have been made from single units in the region just beyond the inner nuclear layer, and on very rare occasions intracellular recordings have also been obtained from these units. The responses have always consisted of relatively slow potentials, with no sign of impulse activity. Although recent work in Svaetichin's laboratory emphasizes the need for extreme caution in identifying receptors, our evidence indicates strongly that these single units are receptors. The responses have proved divisible, with intra-cellular recording and very short stimuli, into fast and slow components which look like the b-wave and DC component. On a number of occasions, intracellular records have also been obtained from cells immediately adjacent to the inner surface of Bruch's membrane. These records show a large c-wave of opposite polarity from the extracellular records.

All of this evidence on localization of different components of the ERG is internally consistent. We interpret our findings to mean that the b-wave and DC component are generated in the cell bodies and/or terminals of receptor elements. The c-wave seems to come from the pigment epithelium, while the a-wave probably comes from the outer segments of receptors."

DR. DONALD B. LINDSLEY (University of California at Los Angeles and Long Beach VA Hospital): "Dr. Brow's comments remind me of some previous work of a similar sort by Ottoson and Svaetichen, and Svaetichen (Act Physiol. scand. 1953, 1956), Brindley (J. Physiol. 1956), Tomita and Toriyama (Jap. J. Physiol. 1956) and most recently Motokawa, Oikawa and Tasaki (J. Neurophysiol. 1957) which suggests that microelectrodes gradually introduced into the retina suddenly produce a large potential change as if a boundary had been penetrated. These results plus other factors of our own experience have caused me to wonder from time to time, whether the b-wave really had anything to do with the retinal elements and/or the bipolar cells. Instead, it has seemed to me, there is a possibility that it might be related to a metabolic or chemical state between the external and internal limiting membranes, or possibly an ionic concentration on one of these membranes. The sudden emergence of a large potential change when a microelectrode penetrates the retina to a certain depth, and the magnitude and widespread distribution of the b-wave potential over the bulbus of the eye together with its resistance to changes which apppear to affect the neuroretinal potentials, suggest such a possibility.

"In this latter connection, we have been trying to localize components of the electroretinogram on the surface of the bulbus, by utilizing a technique of phase reversal similar to that employed in the electroencephalogram for localizing brain tumors and other aberrant phenomena. By placing our electrodes on the sclera around the exposed bulbus, along the horizontal or vertical perimeter, we find we can obtain a phase reversal of the ERG components at some location between the cornea and the back of the eyeball. At first we thought this was in the region of the ora serrata, but it is farther back. Mr. Roy S. Griffiths and I have been investigating this further by recording from small trephine holes in the sclera at intervals around the bulbus, or by sewing wick electrodes to the sclera.*

"If I may, I would like to show a slide illustrating the reversal of phase of the a- and b-waves of the ERG along the vertical perimeter of the bulbus of the cat when the eye was exposed to a moderately bright 10 micro-second flash of very narrow band-width at 490 millimicrons. As may be seen in this slide (Figure 1) the a- and b-potentials recorded from electrodes 7-4 and 8-7 are in the same orientation or in phase. Likewise the potentials of 18-17 and 17-8 are in phase, but both of these are reversed in phase from those of the anterior electrodes. The point of reversal, according to the theory of the phase-reversal tech-

* All work reported here by D. B. Lindsley and R. S. Griffiths was carried out in the Neurophysiology Unit of the Investigative Medicine Laboratories, VA Hospital, Long Beach, Calif. and was supported by a USPHS Grant B-362.

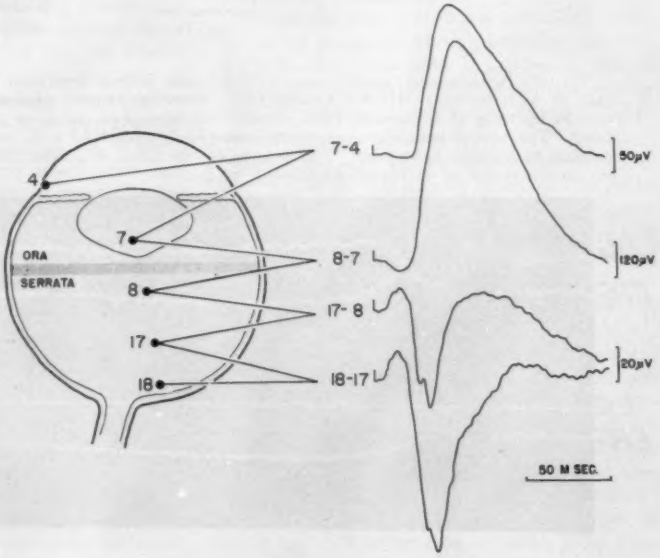


Fig. 1.—Electroretinograms simultaneously recorded from various locations over the surface of the bulbus of the cat, with electrodes arranged to detect point of phase reversal of potentials. A ten microsecond, high intensity flash, of 490 millimicrons, is indicated by vertical line at start of each trace. The a- and b-waves reverse in phase at point 8. (Lindsley and Griffiths, UCLA and VA Hospital, Long Beach, Calif.)

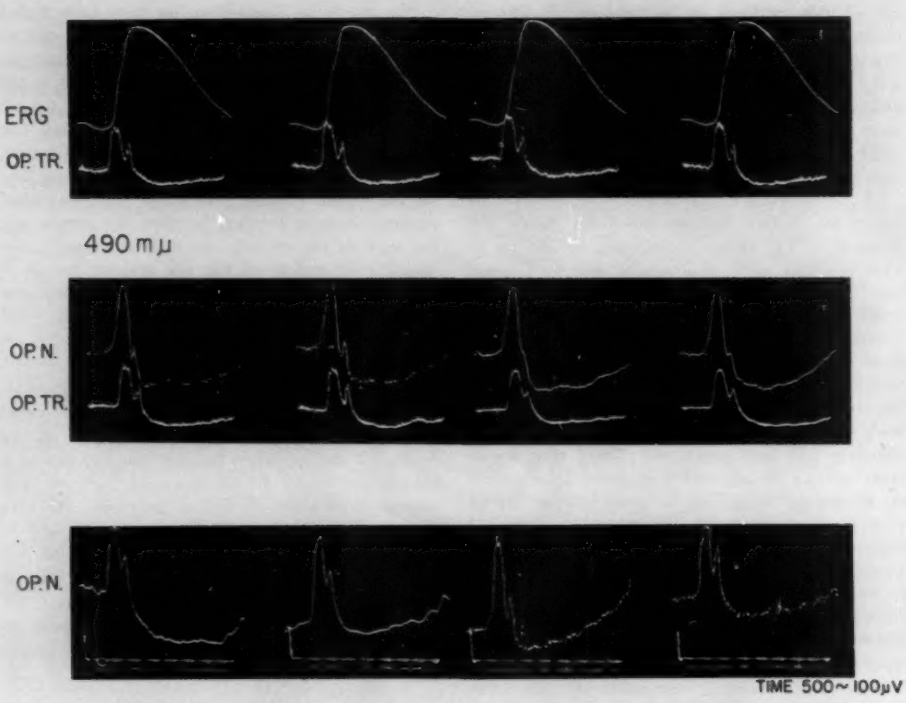
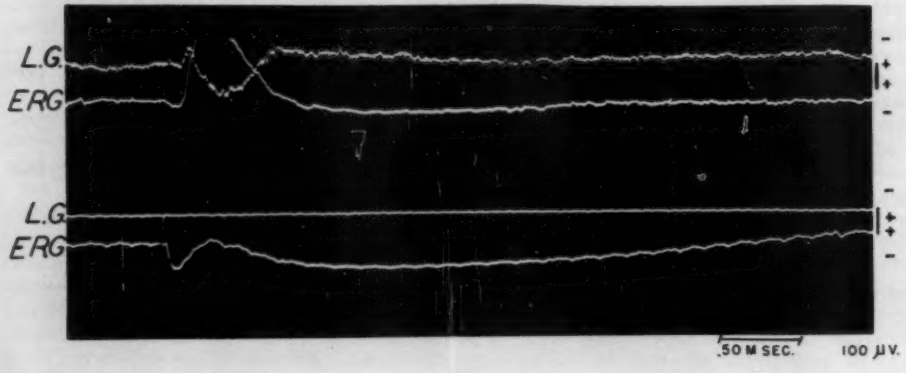


Fig. 2.—Simultaneous oscillograms of ERG and optic tract response, optic nerve and optic tract response, and optic nerve response with signal and time line. Optic nerve and optic tract responses begin before major upswing of b-wave suggesting that the latter does not initiate the optic nerve and tract discharges.

Fig. 3.—Simultaneous oscillograms of ERG and lateral geniculate response to brief light flash of high intensity and 490 millimicrons. Note that lateral geniculate response begins as soon as upswing of b-wave of ERG. Lower tracings after condition of anoxia has been produced. The lateral geniculate response is completely abolished with only a signal artifact remaining to indicate light flash. The b-wave of the ERG is almost abolished, and the a-wave has increased due to decreased antagonism by b-wave.



nique, is in the neighborhood of electrode 8. Along the horizontal perimeter we have observed this reversal point slightly farther back. The a-wave appears to have a shorter latency and be more prominent toward the back of the eyeball; the b-wave is attentuated markedly in both voltage and duration toward the rear of the eyeball (note calibrations for differences in amplification).

I should like to show two more slides to emphasize that the b-wave can hardly be responsible for triggering the optic nerve discharge as Granit once proposed. In the first of these (Figure 2) are shown combinations of ERG and optic tract responses, paired optic nerve and optic tract responses and optic nerve responses alone with the other trace of the oscilloscope showing the onset of a brief, bright, photoflash at 490 millimicrons and a 500 cycle time line. The latency of the optic nerve and optic tract responses will be seen to be as brief as the apparent onset of the b-wave itself, if not actually preceding it. However, it must be recognized that the a- and b-waves are of opposite sign and tend to cancel one another, so that the b-wave may be initiated slightly earlier than it appears.

"The next slide (Figure 3) shows the lateral geniculate response paired with the ERG, and again it may be seen that the response latency at the lateral geniculate is almost as short as that of the upswing of the b-wave. The lower portion of this figure shows the response in the same preparation after a condition of anoxia had been produced. The lateral geniculate response is completely abolished, leaving only the stimulus artifact. The b-wave of the ERG is largely abolished due to the anoxic condition and the a-wave has increased in apparent size due to lack of opposition by the b-wave. The fact that the a-wave is preserved might suggest that it originates in elements less susceptible to anoxia, and since the neural elements of the lateral geniculate failed to respond under this condition of anoxia, it might be speculated that the a-wave arises from non-neural elements such as the cone-receptors. The b-wave might be thought to arise from neural elements, but not the rods, since it is abolished by anoxia as are the waves from the lateral geniculate. Presumably, then, if the rods like the cones serve an excitor function this is not manifested as a b-wave for its latency and duration are too great. It is possible it could be an after-potential associated with bipolar cell or ganglion cell action, but the suggestion made earlier, that it may be associated with an ionic state of the limiting membranes, or a metabolic condition in the region between them should not be overlooked. A number of factors, which cannot be described here, argue against the b-wave (regardless of its origin) being responsible for initiating the optic nerve discharge."

DR. ALBERT M. POTTS (University Hospitals, Cleveland, Ohio): "If one compares the spikes which we obtained from the cat's ganglion-cell layer, and checks the time relationship between these and ERG, one gets onset of the discharge long before there is any b-wave. I do not think there is any question about the relationship of optic-nerve discharge or single fibre spikes to the onset of the b-wave. The b-wave almost certainly has to be considered a correlate of the optic process, but not the actual initiating potential."

DR. JOHN C. ARMINGTON (Walter Reed Army Institute of Research): "I would like to say something about the latency of the b-wave and optic nerve discharge.

"Recently we have been making simultaneous recordings of the electroretinogram and the response from the optic tectum. We find that the tectal response may either precede or follow the retinal response, depending upon the state of the eye's adaptation, stimulus luminance, and other parameters. For example, if the eye is light adapted, and other stimulus conditions are appropriate, the response at the tectum follows the electroretinogram. If the eye is dark-adapted, the reverse situation may hold.

"I think a number of factors must be considered when comparing the electroretinogram with the optic nerve discharge. You have to be sure that the responses from both locations are initiated by the same photopic or scotopic systems. It is quite possible, for example, that the optic-nerve discharge under a particular set of recording conditions could be released by the cones; while the electroretinogram, recorded at the same time, was being produced by the scotopic system.

"I think the role of glare and stray light within the eye is important when considering latencies. We know that stray light is a major source of stimulation for the electroretinogram, particularly when the eliciting light flashes subtend a small visual angle.

"One other point (which may not be too important) is that the first impulse which goes down the or worthy of study. The first big blast which goes down our optic nerve after a bright stimulus may enable us to blink our eyes; but, if we are making a complex judgment or using our eyes for a more normal visual act, perhaps some of the late low amplitude discharge may be important.

"Doctor Crampton, who has been my associate in most of these experiments involving tectal and retinal recording, has also done some experiments in which he has moved an electrode through the vitreous and around the outside of the eye. His results are very similar to those which have been described by Doctor Lindsley and Doctor Noell."

DR. ROBERT M. BOYNTON (University of Rochester): "I would like to make a comment

about a previous question, in connection with the efficacy of large stimuli for eliciting photopic responses. Dr. Riggs, in his talk, said that most of the cones were concentrated in the macular region. I think what he should have said is that the highest *density* of cones is in the macular region. I have a couple of calculations here. If we take 140,000 cones/mm.^{2} as the figure for cone density in the center of the fovea, and assume a figure of about 0.3 mm. as equal to one degree, then we would have on the order of 10,000 receptors which would be located within this region. If you wanted to include, say, four degrees, you would have at the most 16 times that many, or around 160,000 (and this ignores the sharp drop in cone density as you move away from the foveal center).

"My only point is that this is a very small number compared to the 5 to 7 million cones which are found in the retina as a whole."

DR. RIGGS: "Frankly, I do not remember all the questions. I might comment on just a couple.

"With regard to Dr. Boynton's remarks, I think I would certainly accept the fact that we do need the large areas for cone stimulation, because the cones are dispersed through the retina as well as being concentrated in the macular region.

"This is certainly true; how many there are in the macular region depends on how big you call the macula. I do not need to go into that.

"Another little point: Dr. Noell mentioned the fact that a small positive wave creeps into the negative deflection with the typical record. Certainly, this is the case.

"The picture I like to think of is that photopic responses have their negative and positive phases, more or less as we showed on the slide; also, the scotopic responses do, the photopic being typically much faster. For example, if you take an all-cone eye, such as the ground squirrel, you find that there you get both negative and positive waves showing up on the ERG. This is also true of the all-rod eyes which have been used.

"So I think the typical picture is one which does include both negative and positive components for both. There have been some errors in this regard in the early literature, where people thought the a-wave was from the cones, the b-wave from the rods. This certainly is not the case. Certainly, there is a good deal of mixing up of the positive and negative components which are photopic and scotopic in nature. This depends very much on the conditions of stimulation and adaptation of the eye.

"One more point: I would like to second Dr. Noell's comments on the apparatus necessary for recording the ERG. I should have said something about that, too.

"The early apparatus was excusable, of course, because it was early. Now we should be very careful, as he said, about the time characteristics of the recording instrument. We need an instrument that will respond faithfully to both fast and slow components of the ERG.

"The ERG is bad this way. We cannot afford to cheat either way; we have to record fast time components and slow time components.

"Unfortunately many of the records in the literature have not used D. C. amplification; and some of those which have used capacitance-coupled amplification have used slow ink-writers in recording the response. This results is in a serious compromise with quality because both fast and slow types of response wave are lost. Faithful recording you have to achieve by the use of a DC amplifier coupled with photographic registration, typically, of the deflections on a cathode-ray scope."

Present Status of Our Knowledge of Stereoscopic Vision

KENNETH N. OGLE, Ph.D., Rochester, Minn.

Introduction

It is said that no self-respecting biologist would state or imply that the eyes of the primates are in front of the head for the purpose of providing the animal with stereoscopic depth perception. Certainly, however, the fact that the two eyes are so placed, whether by design or by mutation, has provided the most favorable situation for the development of such perception. Even if one believes that the second eye is simply a reserve organ for security and that stereoscopic depth arises as some sort of learned by-product of the dissimilar images in the two eyes due to their location, we still must concur in the statement that stereoscopic depth perception plays an important role in spatial localization. Phylogenetically, the gain in obtaining stereopsis has perhaps been accompanied by a loss of panoramic vision, but much more important, this gain has so very greatly complicated the physiologic processes of vision that a highly complex and intricate sensory and motor co-ordination of the two eyes is required. Stereopsis must then have played an important role in the particular phylogenetic development of the species.

The brightness of objects seems little or no greater with binocular observation than with monocular. Likewise, one finds that visual acuity when both eyes are used is only slightly greater than when one eye is used. The critical flicker fusion frequency differs only slightly between monocular and binocular observation. In binocular space perception, however, there emerges an entirely new sensation that is not in the least suggested by observation with one eye alone.

The stereoscope, invented by Wheatstone [1] nearly 120 years ago, with which different pictures could be presented to each eye alone, demonstrated clearly and decisively the fundamental difference between binocular depth perception and the conception of depth and distance from essentially monocular vision. In figure 1, which should be very familiar, each of the eyes observes patterns consisting of vertical parallel lines, the two patterns differing only in that the lines have different separations. Provided that the difference in separation is not too great, when the images of the lines are observed by the two eyes they "fuse" (and the word "fuse" is used here only to

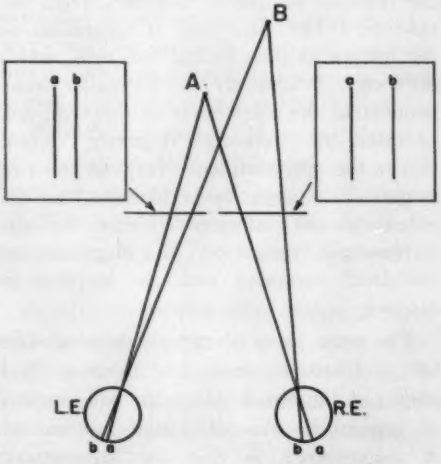


Fig. 1.—Illustration of the fundamental basis for stereoscopic perception of depth.

Read as part of the Symposium on Visual Mechanisms sponsored by the National Institutes of Health, Bethesda, Maryland, September 11, 1957.

Section of Biophysics and Biophysical Research, Mayo Clinic and Mayo Foundation. The Mayo Foundation, Rochester, Minnesota, is a part of the Graduate School of the University of Minnesota.

mean that the observer experiences visually two lines only). There is an almost immediate and certainly a striking spatial experience that one of the lines appears definitely more distant than the other, exactly as if two line objects at different distances in space were observed. Monocular observation of either target gives no hint of this spatial experience. Such figures as drawn for the stereoscope are free from ordinary empirical motives or cues to depth localization, and the striking and impelling sensation of difference in distance with observation by the two eyes constitutes the unique phenomenon of normal binocular vision. In this experience we seem to "see" the space or the "air" between the two objects in depth. So unique is the stereoscopic effect that it cannot be easily described to one who does not experience it. The phenomenon must be experienced.

The positions of the two lines on each of the two targets correspond to the retinal images of two actual lines in space as observed by the two eyes. The difference in the separation of the images of the lines as formed in the two eyes depends on the different points of view of each eye and thus on (1) the amount of separation of the two eyes, (2) the difference in distance of the two line objects in space, and (3) the distance of one of the lines from the observer. The difference in separation of the images as seen by the two eyes, which difference incidentally is essentially independent of the movements of the eyes, will be called the "geometric disparity." Since this is the only difference between the two targets, this disparity is identified as the necessary and sufficient stimulus for the stereoscopic experience. The magnitude of the depth increases with the increase in disparity within certain limits.

The great mass of experimentation with the stereoscope since its invention has played an important role in the development of general theories of spatial localization. A consequence is that the stereoscopic depth has been called the *primary* factor in visual spatial localization, and all other conceptions of depth as obtained from empirical cues and learned associations between known objects and their retinal images have been called empirical or *secondary* factors. This does not imply that the empirical cues are of secondary importance, for probably the reverse is true; the secondary cues represent a higher, typically advanced faculty, in which interpretative processes come into play, to preserve a stable and meaningful spatial perception.

Stereoscopic depth is relative depth perception: object A is farther (or nearer) than object B. Stereopsis alone does not provide the sense of how far away A is from the observer (the perception of so-called absolute distance). To be sure, it has been suggested that if objects associated with the observer, such as the nose or parts of his body or clothing, are also seen, stereopsis in fact plays a role in the perception of absolute distance. The egocentric or absolute distance must result from a complex of many empirical factors and stereoscopic depth differences. It is possible that the perception of the relative stereoscopic depth interval of A and B, if this has to be expressed in terms of inches or feet, demands the participation of central processes and past experience. The stereoscopic depth, as experienced from the images of a number of different objects at different distances in space, alone may indeed provide only a "rank-order" scheme of depth.

Also, if A is the point fixated, then, whether B is seen "nearer" or "farther" depends only on whether the separation of the lines is smaller or greater for the right eye than for the left, that is, whether the disparity is crossed (positive) or uncrossed (negative). However, the "nearer" or "farther" stereoscopic depth response would result, even for two object points of which the image disparities were unequal but were both crossed or uncrossed. In this respect we have an instance of strict psychophysical parallelism in a rank-order sense.

Unfortunately, stereopsis cannot be studied as one might study a purely physiologic process, because the end product, starting with the stimulus of disparity, is a psychic experience. Psychophysical procedures with human beings as subjects must be used, and then the results are described in phenomenologic terms. From these one tries to deduce, within available knowledge of the neuro-anatomic and physiologic structures of the visual system, something about the underlying processes that may be involved. In so far as possible, however, it is more satisfying to strive to substitute in explanations of experimental findings physicophysiologic mechanisms for the vague notions and words that characterize some of the writings to be found in literature. Similarly, in so far as possible, quantitative relationships between the stimulus and the response are highly desirable. The more precise the data, the more satisfying is the general knowledge of the subject. On the other hand, there must be concern with the psychic or experiential influences and variables that exist in ordinary vision, and which may enhance, inhibit or modify the simple experience of stereoscopic depth which might otherwise be obtained if the stereoscopic stimulus could act alone. In the main the concern is with the stability of the relationships between the experience of stereoscopic depth and the characteristics of the disparate stimuli.

Brief Résumé of Current Experimental Evidence

I. Contours.—In the first place, the importance of contours, the boundaries that demarcate the light and dark areas and which delimit or define objects to be seen in depth, must be stressed. Experiments in which the figures in the stereoscope exhibit retinal rivalry show the persistence and dominance of contours over the interior areas. It is the disparity between the retinal images of these contours, and not the similarity or identification of the objects themselves, that is the stimulus for the emergence of stereoscopic depth. This fact is brought out by the figures of Helmholtz [2] (reproduced in fig. 2), in which the contrast of one of the two figures to be observed in the stereoscope was the reverse of that of the other. Yet the outlined figures are seen in stereoscopic depth, in spite of some rivalry between the backgrounds of the two figures. The two drawings also may be of different color, but the contours surrounding the figure and background will determine the stereoscopic experience. The success of stereoscopic depth from the use of anaglyphs, which is a method of separating the images in the two eyes by means of color differences, depends on this fact.

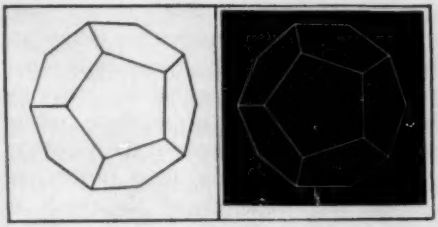


Fig. 2.—Figures from Helmholtz which show the importance of contours in the perception of stereoscopic depth.

II. Fusion and Double Images.—Fusion of the disparate images is not a prerequisite to the experience of stereoscopic depth. Experiments conducted under carefully controlled circumstances show that the disparity may be so large that the disparate images are seen double, but even so, both images then participate in and contribute to a depth experience. However, the disparities can be too large, for beyond certain limits of disparity stereopsis in the usual sense no longer exists, and the two half-images are finally indefinitely localized in space.*

Experiments [3] with large disparities give evidence that the character of stereoscopic depth perception changes as the disparity between the images is increased, and that there may be two phases or even types of

* This fact is one argument against a purely Gestalt explanation of stereopsis.

stereoscopic depth. Within a certain range of small disparities, whether the images are fused or not, the depth experience is patent and compelling. Also within this range the subjective magnitude of depth is related definitely to the magnitude of the disparity.[4] As the disparities are then further increased, the magnitude of the depth no longer appears to increase with the increase in disparity, and the relationship between subjective magnitude of depth and the disparity becomes less definite. On the other hand, while there is never any confusion or question as to whether the participating half-images, appearing as a configuration, were farther or nearer than the fixation point, it is difficult to sense *how much* farther or nearer. Finally, with further increased disparity, even this sense of depth gradually fades and finally vanishes.

It is convenient to characterize the first phase of stereopsis that occurs with small disparities as an "obligatory" response to the disparate stimuli, that is, it is compulsory and always of the same character, and does not change its nature with varying conditions; it is always a depth effect. Likewise, it is convenient to characterize the second phase that occurs with large disparities as a qualitative response of "nearer" or "farther" which is more susceptible to changes in external circumstances that may alter its character. The qualitative depth perception is less evident with continuously observed images, especially if no eye movements are made. Eye movements, then, appear to enhance the qualitative experience of depth, especially in surroundings in which binocular parallax[5] and empiric clues are prominent. One would be led to believe, from these experiences, that the qualitative depth perception may rest on empiric bases. Perhaps related to qualitative depth is the result obtained in recent experiments[6] in which half-images located with very large disparities—that is,

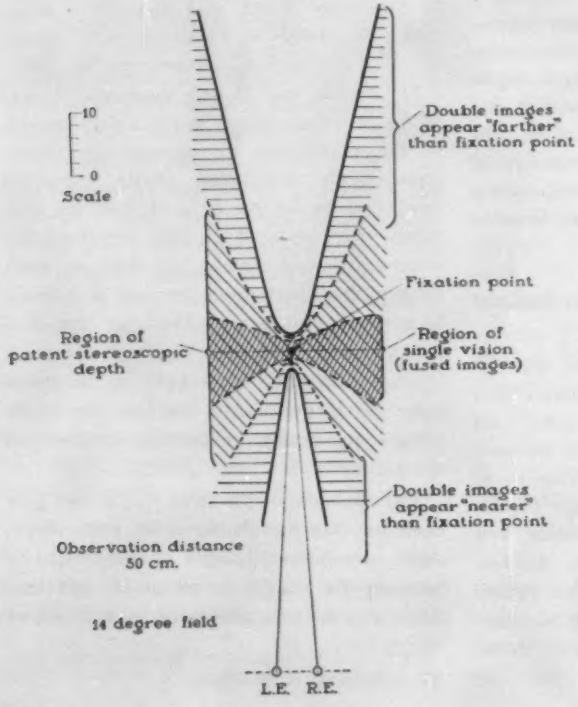


Fig. 3.—The regions of obligatory and qualitative stereoscopic perception of depth about the point of fixation.

of the order of several degrees—are presented briefly. When subjects were asked to guess whether those images corresponded to an object that was nearer or farther than the fixation point, it was found that some had a far higher percentage of correct guesses than of wrong ones. In this there were large individual differences.

Figure 3 shows approximately the spatial areas about the fixation point (at an observation distance of 50 cm.) corresponding to the fused images, the obligatory and the qualitative phases of stereoscopic depth. These results can be accounted for in the same manner as can be Panum's areas of fusion (the limits of disparity within which the images are fused or seen single), namely, by the extent of the overlapping of the arborizations of neurons from corresponding retinal points in their terminal areas in the cortex.

It has been reported [7] that, by a suitable arrangement of mirrors, stereoscopic depth has been observed in those peripheral areas of the retinas of the two eyes in which binocular vision always is prevented because of obstruction of the field of view by the nose.

III. Stereoscopic Acuity.—Probably the one single type of measurement, and that which is most readily available, for the study of stereoscopic vision has been the obtaining of stereoscopic thresholds, or the inverse, of stereoscopic acuities. The greatest amount of data reported in the literature pertains to stereoscopic acuity, as obtained under a great variety of conditions. The threshold of stereoscopic depth will be the smallest difference in depth, and therefore the smallest disparity, that can be discriminated. Like other psychophysical thresholds, this must be specified as a quantity obtained statistically. The best procedure is to use the psychophysical method of constant stimuli, from which the frequency of correct responses to a series of equally spaced disparities can be obtained. The threshold then may be expressed as the standard deviation of the integrated normal curve that describes those data, or it may be expressed in terms of some arbitrarily agreed upon percentile of the frequency of correct responses. It has also been assumed that the standard deviation (and even mean deviation) of the errors in settings also are proportionate to a psychometric threshold. The differences in the methods of specifying the threshold have been a source of confusion when values reported in the literature have been compared. The stereoscopic threshold will vary with the type of test used and with the conditions under which it is administered.

A. Relationship to Visual Acuity: Stereoscopic thresholds will depend on many specific factors, not the least important of which are the dimensions and the contrast sensitivity of the receptor elements of the retinas, and the sharpness of focus of the optical image of the retina—that is to say, the size of the blur circle. Since the visual resolving power and, therefore, the visual acuity also will depend on these same factors, a correlation between stereoscopic and visual acuities could be expected. Study of the literature shows much conflicting evidence, but in a general way it can be said that the poorer the visual acuity, the poorer the stereoscopic acuity. This correlation is more likely to be found when the visual acuities are worse than or are below 20/20 Snellen. For subjects whose acuities are keener than 20/20, however, the correlation generally has been poor. However, as a general rule, except for one study,[8] different types of test details have been used to test the two functions, so that a lack of a significant correlation should not be too surprising.

But in the same person, if peripheral angle is considered, the decrease of stereoscopic acuity in the periphery appears to follow quantitatively the decrease of visual acuity with peripheral angle.[9-11] In this regard, however, there is some evidence that the stereoscopic acuity is maximal, not for

the foveas, but at an extrafoveal angle of about 15 to 21 minutes of arc.[12,13]

The correlation of stereoscopic acuity with the degree to which the optical image on the retina is blurred appears to be more complicated than one might suspect. There appears also little doubt that the response of the normal person to a blurring of the retinal images with ophthalmic lenses is not the same as the response of the person who normally has the equivalent uncorrected refractive errors. Experiments reported in the literature in which stereoscopic acuity has been measured when the images were blurred with ophthalmic lenses also have brought forth a variety of results.[14,15] A recent study shows that equal blurring of the images in the two eyes, if not too great, may result in only a small decrease in stereoscopic acuity over that with the sharp imagery.[16] This is due in part to the fact that the "contours" of the blur circles of images, being nearly the same, act as fairly sharply defined contours for the stimulus to stereoscopic depth. Only when the blurring of the images in the two eyes is unequal will a loss of stereoscopic acuity be found, although this also is not an invariable rule, for again, much depends on the characteristics of the two blurred images.

It is probable that the blurring of the image on the retina is not a simple matter, but rather, because of the irregularities of the various refractive surfaces of the eye and because of the variations of the indices of refraction of the crystalline lens, there are separate small concentrations of light at various places within the blur circle. These small spots of increased light intensity may provide "contours" which, so far as the retina is concerned, may supply one of the half-images of a stereoscopic stimulus. These results imply that a person's stereoscopic acuity under suitable conditions might be better than his monocular visual acuity.

Stereoscopic acuity depends, of course, to a certain extent on the nature of the test stimulus. The greater the number of retinal elements brought into play by the images of the test stimuli within certain limits, the better the stereo-acuity.

B. The Limiting Distance of Stereoscopic Depth Perception: There has always been considerable interest in the fact that associated with a threshold of stereopsis there must also be a distance beyond which stereoscopic depth perception cannot operate. This limiting distance can be computed readily, given the disparity threshold and the separation of the pupils. Because the stereoscopic threshold is a statistical quantity, however, such a limiting distance never can be stated precisely. Experimental determination of this limiting distance is difficult because, as the range of limiting distance is approached, the empirical factors in depth perception become relatively stronger, which complicates the problem. Much depends on the nature of the test details and of the terrain upon which the tests are made, as well as on the method used to conduct the experiment. Early studies with a pseudoscope [17] placed the limiting distance at about 580 meters, which corresponds to a disparity angle of about 24 seconds of arc. More recent field studies [18] suggest a limiting distance of about the same magnitude. In the laboratory, of course, stereoscopic thresholds considerably less than 20 seconds of arc have been measured. In the use of the stereoscopic range-finder 12 seconds of arc has been considered the unit of error. Hence, depending on the nature of the objects in the field of view and on the terrain, it is probable that at times some assistance from stereopsis occurs, perhaps only momentarily, at even greater distances.

Some evidence is reported in the literature that the threshold of stereopsis may decrease, within certain limits, with increase in observation distance.

C. Relationships of Stereoscopic Acuity to Illumination: 1. Adaptation level. Just as visual acuity changes with the level of adaptation of the eyes to light, so also

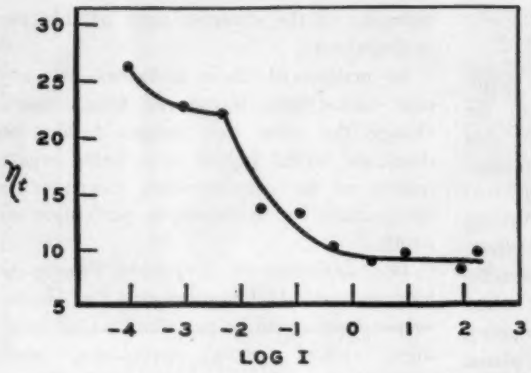


Fig. 4.—The threshold of stereoscopic perception of depth (in seconds of arc) as a function of adapting luminance. (Data of Mueller and Lloyd, used with permission of the National Academy of Sciences.)

would one expect stereoscopic acuity to increase with increase in the luminance of the adapting background. Indeed, this has been shown to be the case.[9] When a three-rod type of test seen in silhouette in a stereoscope arrangement is used for levels of background illumination from 10^{-4} to 186 millilamberts—that is, from low scotopic to high photopic levels of adaptation [19]—the behavior of the threshold for stereoscopic depth with increased luminance (fig. 4) resembles the cone-and-rod components of the dark-adaptation curve. The data show that the threshold decreases (the stereo-acuity increases) as the luminance increases, until at high intensities the curve approaches a limiting value asymptotically. At low luminances, in scotopic vision, the usual discontinuity between the rod-and-cone functions appears. In this range of illuminations, however, there is great individual variability. Nevertheless, it is clear that stereoscopic vision occurs with either cone (photopic) or rod (scotopic) vision.

2. Contrast. For a constant level of adaptation to light, corresponding to the luminance of the background, recent experiments [20] have shown that the stereoscopic depth threshold is independent of the contrast of the test detail—that is, of the ratio of the luminance of the detail compared to that of the background. Changes of luminance of the test detail, even over a range of 250 times, did not significantly change the stereoscopic threshold. One concludes, therefore, that if the test details are visible (other factors being constant), the stereoscopic threshold would depend only on the luminance of the background, that is, on the retinal light adaptation level.

3. Unilateral darkening of the image of one eye. It has also been shown [21] that changing the contrast of the image to one eye only, in the case of static test objects (unilateral darkening)—one image bright, the other less bright—has little or no effect on the stereoscopic acuity or on the magnitude of the subjective stereoscopic depth. So long as both the half-images of the stereoscopic test-line detail are seen by the two eyes, at a constant level of background (adapting) luminance, stereoscopic depth

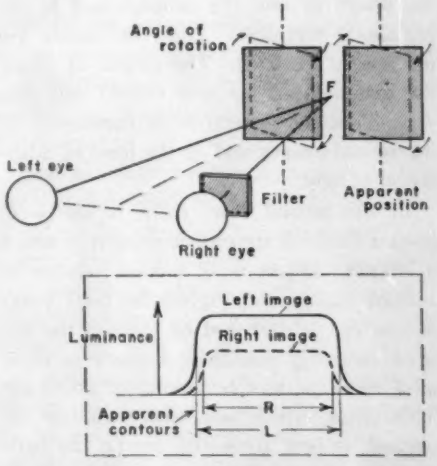


Fig. 5.—Experimental arrangement (due to Cibis) to illustrate irradiation stereoscopy.

remains valid, and the stereoscopic acuity is reasonably constant.

The magnitude of the stereoscopic depth must be carefully distinguished from the stereoscopic acuity or precision of that depth. With regard to the apparent magnitude of stereoscopic depth there are two special situations in which the darkening of the image in one eye has a marked effect. In the first, which might be called "irradiation" stereoscopy,[22,23] two squares of white cardboard, on either side of a fixation point, both in the objective frontoparallel plane, will appear as though rotated through a small angle equally and in the same direction about vertical axes (a Venetian-blind effect) when the image of one eye is darkened by a filter (fig. 5). The effect is simply due to the phenomenon of irradiation, in which the position of the perceived contour from the retinal image of a sharp boundary between light and dark areas varies with the respective luminances. In the present instance, the effective size of the squares as seen by the darkened eye is smaller than that seen by the undarkened eye. The apparently rotated position of the squares is then explained on a simple geometric basis of the apparent rotated positions of objects about a vertical axis when the image of one eye is magnified in the horizontal meridian. Thus, the cards are incorrectly localized. The extent to which the card appears to have turned will then depend on the difference in luminosity of the retinal images and on the level of adaptation to light.

In the second case, there is the well-known Pulfrich stereophenomenon in which a stimulus object is in motion relative to a fixed background. When the total image in one eye is darkened by a filter, the bob of a swinging pendulum appears to move in a more or less horizontal elliptic path. This effect, which has been variously explained, is best accounted for on the basis that the reaction time of the physiologic process of one eye is increased when the intensity of the stimulus light of that eye is decreased.

In neither of these instances has any new factor been introduced which would change the view that images falling on disparate retinal points in a stable organization of the corresponding elements are the stimuli for stereoscopic perception of depth.

IV. Influence of Exposure Time.—As long ago as 1841 Dove reported that stereoscopic depth could be perceived when drawings, viewed in a stereoscope, were illuminated solely by the light flash of very short duration from an electric spark. Similar tests, including the Hering falling-bead test, have verified this result. These experiments have been cited as evidence that neither convergence movements of the eyes nor proprioceptive influences from the external muscles of the eyes are necessary for the emergence of a stereoscopic perception of depth.

Only since 1926 [24] have systematic experiments been performed to find the relationship between stereoscopic acuity and the duration of the test stimuli. Many of these experiments were faulty in that the conditions of test were not held constant, or in the fact that the test was made too complex. Recent data [20] have been obtained in which all parameters, and in particular the adapting luminance and the angle between fixation point and test object, were held constant. Standard psychophysical procedures were used to determine the stereoscopic thresholds when only disparity was

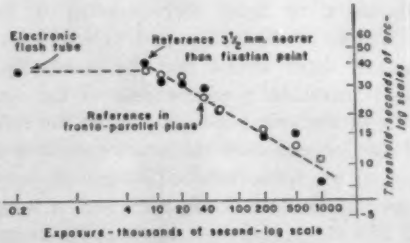


Fig. 6.—The relationship between threshold of stereoscopic perception of depth and duration of the exposure of the test details.

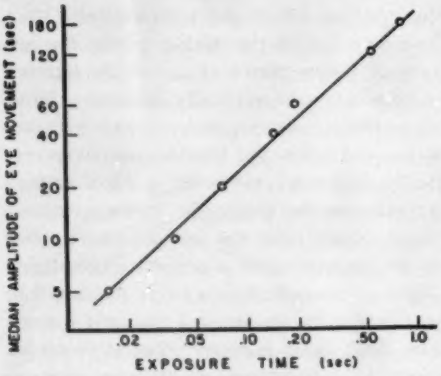


Fig. 7.—Mean excursion of eye movements as a function of the observation exposure. (Data of Riggs, Armington and Ratliff.)

varied. With a range of exposure time from 1 second to 0.0075 second (7.5 milliseconds), and one exposure time of 0.0002 second (0.2 millisecond), the data showed that the stereoscopic thresholds slowly and then more rapidly increased, as the exposure time was decreased down to about 0.005 second (5 milliseconds), after which the threshold tended to remain constant for further decreased exposure time. Figure 6 illustrates results as plotted on logarithmic scales. On these scales the data for exposures from 1 second to 0.005 second (5 milliseconds) with a fourfold increase of stereoscopic threshold appear to be described by a straight line. Further experiments showed that this relationship was essentially the same even when the test and reference lines were observed in a fixed amount of crossed and uncrossed disparity.

This decrease in stereoscopic acuity with reduced exposure time is best explained by the role played by the normal ocular nystagmoid or tremor movements of the eyes. These movements are to a certain extent random or at least aperiodic. The smaller, involuntary tremor movements are independent for the two eyes, have varying frequencies up to about 100 per second, and have correspondingly varying excursions with a median of about 15 seconds of arc. These movements cause proportionate movements of the image on the retina, so that the images of observed objects actually will be continuously moving over a number of retinal elements. The shorter the exposure, the less will be the average extent of the excursions which would occur in short intervals of time. Figure 7 shows the mean eye excursion [25] plotted against the exposure time, both on logarithmic scales. The data, which appear to be described by a straight line, are to be compared with the data on stereoscopic thresholds shown in figure 6, which cover the same range of exposures. On the basis of this explanation, stereoscopic acuity is related in some quantitative manner to the number and extent of the excursions of these involuntary eye movements that have taken place during the exposure of the test stimulus. For very short exposures of less than about 5σ, the retinal image is "stopped," and hence an upper threshold of stereoscopic vision would be expected. This is all consistent with the evidence that these movements, within limits, normally improve visual acuity.

V. Vertical Disparities.—Since the classic experiments of Hillebrand,[26] it has been generally accepted that vertical disparities between the images in the eyes of object points in space do not give rise to a specific stereoscopic perception of depth. No subsequent experiments have disproved that conclusion.

This problem is different, however, from the one that seeks to answer the question: What is the effect on the stereoscopic response from transverse disparities when one introduces also a vertical disparity? Recent experiments [27] in which a point light source was used as the test object show that vertical disparities of considerable magnitude indeed can be introduced between the images of the point source before stereoscopic vision is abolished. The type of results obtained are shown in figure 8, in

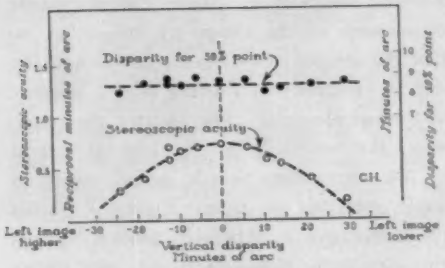


Fig. 8.—The relationship between stereoscopic acuity and the vertical disparity introduced between the images of the test detail. Also shown are the data for the validity of the stereoscopic depth.

which the abscissa is the vertical disparity introduced and the left ordinate scale is the stereoscopic acuity (reciprocal minutes of arc); the right ordinate scale shows the most probable disparity corresponding to that transverse disparity for which the test object appeared the same distance as did the fixation point. This graph shows that the validity as indicated by the upper set of data is stable over the range of vertical disparities introduced. The stereoscopic acuity only slowly decreases for increasing vertical disparities, but even for vertical disparities of 25 to 30 minutes of arc there is still some stereoscopic perception of depth.

The unexpected result is that relatively large vertical disparities, even for test points near or over the point of fixation, are necessary before stereopsis ceases. With those vertical disparities the images actually will be seen as being vertically double, and still the two half-images act as a single configuration and a stereoscopic depth is sensed.

In the normal use of the eyes in one's usual surroundings, only object points close at hand, and only those above or below the visual plane, can give rise to images which can be vertically disparate. These images are then vertically disparate because the distance from the object point is greater to one eye than to the other, and hence the point subtends a smaller vertical angle to one eye than to the other. Even then, if the eyes are converged symmetrically by a fixation point in the median plane, the images of points above or below the fixation point will not be vertically disparate. With asymmetric convergence, those images above and below the fixation point are vertically disparate. However, vertical disparity between the images in the two eyes of object points near the fixation point either never (except when a vertical fixation disparity is present) exists or is too small to be effective in the normal use of the eyes. The data show, however, that stereoscopic depth is experienced when large vertical disparities are introduced between the images of objects near the fixation point. This cannot be explained as the result of localized learning from past experience. The neuro-anatomic structures must be present, although such structures had never before been utilized in this unusual manner.

Now it must be kept in mind that a general introduction of vertical disparities of the images from two or more vertically separated contours in the visual field does have an over-all effect on the apparent orientation of the field of all objects as perceived by stereoscopic localization. When the eyes are fixated in asymmetric convergence for a near object, or when an afocal meridional magnifying lens is placed before one eye at axis 180°, which introduces a pattern of vertical disparities for all object points, there is an apparent re-orientation (the induced effect)[5] of all objects in the entire binocular visual field about a vertical axis at the point of fixation. This visual phenomenon, in which the localization of all objects in the binocular field of view is affected, must be carefully distinguished from the fact that a vertical disparity between the specific images of an object point has little effect on its own specific stereoscopic localization as sensed through the stimulus of transverse disparity.

VI. Simultaneous Stimuli.—For a stereoscopic experience of depth to emerge from transverse disparate images, is it necessary that the stimuli be received by

the two eyes at the same time—that is, must the two stimuli be simultaneous? The term "simultaneity" cannot be taken literally, because stereoscopic depth can be experienced within limits from disparate images presented successively first to one eye and then to the other.[28] Probably, under such conditions, the experience results from a persistence of vision, or from after-images. Repeatedly, it has been shown that stereoscopic depth can be experienced from disparate after-images induced in each eye separately, although the sensation from such images apparently lasts only so long as the after-images themselves persist.[29] In this respect, then, stereopsis is experienced only so long as the disparate retinal elements stimulated by the after-images are in an excited state at the same time.

In experiments in a haploscopic type of apparatus and in which the Hering falling-bead was used as the test object,[30] baffles were so arranged that the upper part of the path of fall could be seen by one eye only, and the lower part of the path of fall could be seen by the other eye only. The baffles were so adjusted that a central portion of the path of fall of the bead could be seen by both eyes. With the eyes directed to a constant point of fixation, the stereoscopic thresholds and the mean disparity were determined corresponding to the subjective criterion that the falling bead would appear the same distance as a reference line. Figure 9 shows the results of the experiment as the gap between the baffles through which the path of fall was seen binocularly was decreased, and then as the baffles overlapped so that there would be a space in which neither eye saw any of the path of fall. The data show that, while there was some stereoscopic sense, although of a reduced amount, after the gap closed, the depth perceived was not valid. This was taken as evidence that for an obligatory patent and valid perception of depth the disparate images in the two eyes must be seen at the same time (this to the exclusion

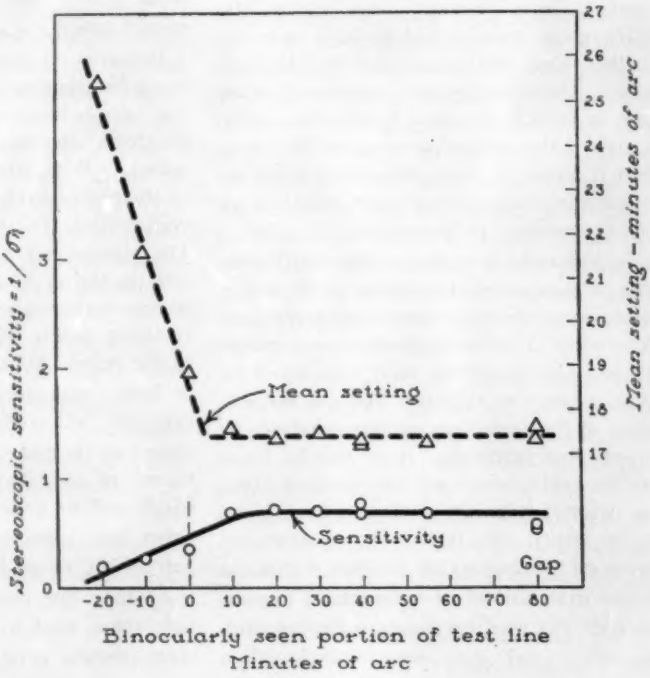


Fig. 9.—Stereoscopic acuity and the mean settings for the Hering falling-bead test, as the portion of the fall seen binocularly is decreased.

of any persistent after-images). The somewhat vague stereopsis experienced after the closure of the gap might be classified as "qualitative," in accordance with the suggestion made in the preceding discussion.

VII. Role of Proprioception.—The experimental work up to 1936 had shown rather convincingly that changes in convergence and changes in accommodation provide most unreliable cues to spatial localization, and consequently could not provide a basis for stereoscopic perception of depth.[31] In spite of the more recent discovery of muscle spindles in the extra-ocular muscles in man and in certain animals, the position is still maintained that "the proprioceptive sense of the eye muscles must be relegated to a vague orienting of the position of the eyes to the position of the head, with an increasing greater effect as it is reinforced by other reflexes."[32]

Many physiologists and ophthalmologists hold the contrary opinion, however, namely, that such a myosensory influence must exist. Chevasse is said to have believed strongly in a "tension sense" from the extra-ocular muscles and to have believed, further, that this sense may be inhibited unless reinforced by the presence of other cues or stimuli to spatial localization. Now if indeed this muscle sense is so inhibited, then it would be most difficult to detect or to measure experimentally the influence of this myosensory process alone.

Experiments have shown that the thresholds of stereoscopic perception of depth are much lower when eye movements were used than when a constant fixation was maintained. This result has been interpreted by some writers as evidence that the convergence of the eyes plays an important role in stereoscopic perception of depth. In these studies modifications of the standard Hering two-rod test were used, and the stereoscopic acuity was tested for a series of increased separations of the two test rods. In the first method of observation, one of the rods was used for constant fixation and the other rod was seen extrafoveally.

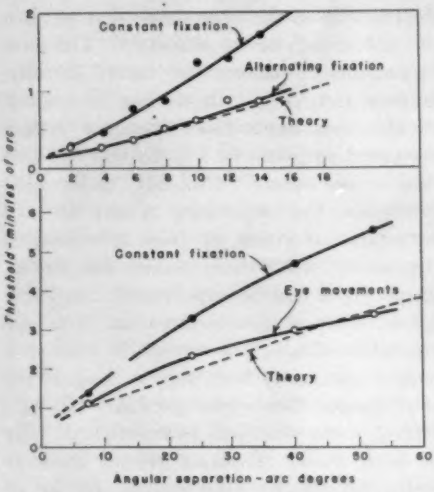


Fig. 10.—The thresholds of stereoscopic perception of depth obtained with and without eye movements, for increased separation of the test details.

In the second method, the first and then the second rod was fixated successively. Figure 10 illustrates graphically typical results. Recent carefully controlled experiments[33] show indeed that the threshold is lower when fixation eye movements are permitted.

However, if the underlying physiologic bases for these results are to be understood, the peripheral visual (and stereoscopic) acuities of the two eyes must be taken into account. With fixation maintained on one of the test objects, the images of the other object would fall peripheral to the foveas. The stereoscopic acuity for the other rod seen in the periphery would depend, then, on the stereoscopic acuity and probably the resolving power of that particular portion of the retina. When the eyes are permitted to look from one rod to the other, both eyes see both rods continuously, and the images of the rods will fall on various parts of the retinas, the stereoscopic acuities of which will be lower than the acuity of the fovea, but higher than that of the peripheral part of the retina involved when one point is fixated. The images of one or the other rod would tend to fall on, or nearly on, corresponding points, because of the fusion

compulsion. At an intermediate point between the two separated test objects, the images of the two objects in the two eyes will fall on portions of the retinas where the stereoscopic acuity will be maximal. One would expect that the stereoscopic acuity measured with alternating fixation would correspond to that stereoscopic acuity obtained with constant fixation at one half the visual angle subtended by the two objects. This would represent the maximal stereoscopic acuity possible due to the stimulus of retinal disparity alone. The explanation implies also that fusional movements will occur to eliminate the disparity of the half-images of the rod nearest the point of convergence of the eyes during the eye movements from one rod to the other.

In the graphs shown, a "theory" line has been drawn where the stereoscopic thresholds found from the constant fixation data are plotted at *twice* the angular separation of the test objects. Inspection shows that the data obtained with eye movements approach this line or actually lie above it. If the small convergence changes—that is, proprioceptive or position-sense impulses from the extrinsic muscles—contributed quantitatively to the stereoscopic acuity, then one would expect the stereoscopic thresholds to be lower than those predicted from the maximal stereoscopic acuity at one half the separation of the test objects. These results suggest, then, that a stereoscopic sense of depth from convergence changes does not occur. Even granting a muscle sense sufficiently precise to play a role in stereoscopic acuity, a sensitivity at least equal to the stereoscopic depth sense of the retinas would be necessary.

Of importance also are the unequivocal findings, in a number of studies, that heterophorias do not affect stereoscopic perception of depth.

The conclusion that a proprioceptive sense plays no role in stereoscopic depth does not preclude the possible assistance given by a myosensory process in the distant localization involving large differences in convergence, nor does it preclude the possible existence of proprioceptive reflexes and "feed-back" innervations which may be an aid in the executing of accurate fixation eye movements.

VIII. Validity of Stereoscopic Depth.—It is a well-known fact that the magnitude of the subjective stereoscopic depth interval corresponding to a given angular disparity increases very rapidly (as the square) with the observation distance. Even for a given observation distance, it can be shown analytically that equal subjective depth intervals farther and nearer the fixation point require that the corresponding uncrossed angular disparity be a little smaller than the corresponding crossed disparity. Yet, careful measurements obtained for a given observation distance with constant fixation on one point show that this relationship indeed is adhered to,[4] and our subjective stereoscopic depth interval is precisely related to that disparity required by the geometry involved.

The problem to be faced is: How is the subject able to relate these disparities so that the valid stereoscopic depth will be experienced? If there was a fixed pattern of disparity for a uniform depth scheme associated with a certain observation distance, this pattern would have to change for other observation distances. Hence, it would appear that the subject must know the observation distance in order to set the pattern if valid stereopsis is to be had generally. This problem then concerns total spatial location in the sense of an absolute localization of distance.

IX. Gestalt Point of View.—From time to time a paper appears in the literature in which certain introspective observations are described, and from these the authors purport to show that stereoscopic depth arises, not from disparity between the images, but rather from the synthesis of the separate Gestalt patterns received through each of the eyes.[34,35]

Two examples (and these may not be representative) of typical drawings, that

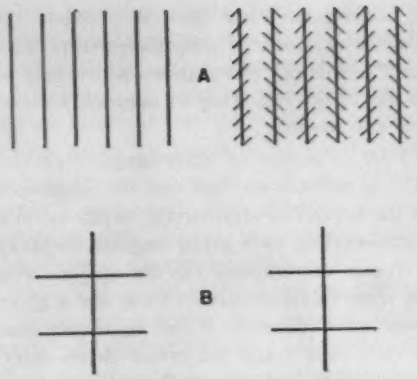


Fig. 11.—Representative types of targets to be observed in a stereoscope and used to illustrate the possible influence of Gestalt factors in stereoscopic perception of depth.

are to be viewed in the stereoscope, are shown in Figure 11. In the first (*A*), a series of vertical, equally spaced lines is seen by one eye. Seen by the other eye is an identical pattern to which have been added the cross-hatched lines which then make that pattern conform to the well-known Zöllner illusion (these two figures often are presented at an oblique angle). The illusion is that the lines appear alternately inclined in directions opposite to each other. It is claimed that,[36] when these targets are viewed in the stereoscope, the fused vertical lines now appear to lean alternately fore and aft, top toward and top away from the observer, as would be expected in stereoscopic depth if the lines of the right target actually were alternately inclined. It is concluded that stereoscopic depth can be obtained without there being actually horizontally disparate stimuli. This is to say that *apparent* horizontal disparities between configurations are sufficient for the emergence of stereoscopic depth experience. Finally, it follows that because of such "Gestalt disparation" some sort of a "configurational" theory is required for the adequate understanding of stereoscopic depth.

Now, the writer has been particularly unsuccessful, as have been some other workers in this field, in experiencing this phenomenon. Apparently, it is possible for one to see in these introspective-type experiments what one wishes to see. Moreover, it is possible that the apparent stereoscopic-depth effect is in fact only a monocular illusion of depth, a suggestion made even by one of the adherents of the Gestalt point of view.[37]

A second type of introspective observation[38] is made with targets as shown in *B* of Figure 11, which also are to be observed in the stereoscope. The horizontal lines of the target seen by one eye are a little longer or shorter than the corresponding lines seen by the other eye. Hence, there are actually transverse disparities between the images in the two eyes of the ends of these lines. Accordingly, the lines appear slightly rotated, the right side of the upper line appearing farther away and the left side of the lower line appearing farther away. The argument is that, because it is the "whole" line in each case that appears rotated and not simply the ends, the spatial orientation is a matter of the "disparity of the figure" and not of the disparate retinal elements stimulated by the images of the ends of the lines.

The more conventional point of view would insist that, (1) because the interior parts of these lines are not a series of discrete points, and (2) if their ends were not seen, the fused images would appear indefinitely localized in space. The normal stereoscopic depth from the disparities of the ends of the lines, then, anchors the lines in space, since there are no opposing binocular or empirical cues.

Although one cannot disregard the many interesting and at times striking results of these and similar introspective experiments, it would seem that the Gestalt interpretations are strongest, and possible, perhaps, only when the physiologic excitations permit more than one interpretation. Then even spatial inversions are possible. It has been insisted that the "Gestalt interpretation cannot be regarded as a regular, let

alone, the primary cause of stereoscopic depth perception." [39]

X. Role of Empirical Factors.—In one's normal spatial localization in familiar surroundings, the empirical factors in that localization play a dominant role. These factors, psychologic in nature, and for the most part concerned with monocular processes of vision, consist of learned association between retinal patterns and events with objects in space that have had meaning to the individual—a meaning acquired during his past experience. Any one of these empirical factors, in the absence of supporting cues, may be ambiguous or at least unstable. Stereopsis as a separate factor, in the totality of spatial localization, acts physiologically and immediately upon the stimulus of disparity, and it would be therefore less concerned with meaning. In ordinary normal surroundings, the empirical cues and the stereoscopic perception rarely conflict, and in binocular observation the spatial orientations of objects in space are more clearly correlated with the actual positions of those objects.

Only in artificial situations, in which the empirical cues to spatial interpretation conflict with the stereoscopic localization, is the latter ever overcome or suppressed, because the empirical physiologic factors are always interpretative, since they are based on a meaningful experience, while the stereoscopic depth, on the other hand, emerges automatically with less regard to meaning or interpretation. Thus it is that the human face, when viewed through a pseudoscope which interchanges the right and left views, still appears convex, although from the disparities between the images one would expect it to appear concave. These general facts regarding the roles of empirical and stereoscopic factors in spatial orientation were clearly brought out in the experiments in which a meridional afocal magnifying lens was worn before one eye for a prolonged period.[40] However, stereoscopic depth is not necessarily suppressed in the presence of conflicting empirical cues, and in many situations it actually dominates the total localization.[41] Much depends on the complexity of the cues and the extent to which they themselves may be ambiguous. Great individual differences are found in this respect.

There is evidence that the psychologic mental "set" or expectancy will affect the perception of depth. In critical tests of stereopsis, and especially those involving short exposure, strict attention and anticipation on the part of the subject is necessary. It appears also that, in the binocular observation of a number of objects in the field of view, eye movements facilitate the perception of stereoscopic depth. Under artificial conditions of observation, stereoscopic depth sometimes fails to develop or will develop only slowly, especially when the disparities are large, as is often the case with drawings to be viewed in the stereoscope. In this regard there are wide individual variations, and much probably depends on the extent to which the individual is accustomed to rely on the empirical factors. Stereoscopic depth sometimes will fade with continuous staring at the pattern, a phenomenon, however, that occurs with other visual phenomena.

Nature of Stereopsis

Review of the experimental evidence presented in the foregoing pages would seem to provide a rather consistent picture of the phenomenon of stereopsis. Disparity and disparity alone provides the necessary and sufficient stimulus for the emergence of the stereoscopic experience, and the validity of this experience implies a stable organization of corresponding retinal points.

In summary, it is clear that it is the images of contours that provide the retinal stimuli, that stereoscopic acuity is consistent with visual acuity in the periphery and more generally, if we take into consideration the complexity of the optical image on the retina, that the change in stereoscopic acuity with light-to-dark adaptation is consistent with the change in visual acuity

(and contrast sensitivity), that for a given level of retinal adaptation stereopsis is independent of the contrast of test stimulus, that unilateral change in contrast does not affect the acuity or validity of the depth experience (with two particular exceptions), that the change in stereoscopic acuity with short exposure times is consistent with the normal physiologic tremor movements of the eyes, that fusion of the disparate images is not a prerequisite for stereopsis, that simultaneity of a special kind is necessary for the valid stereoscopic experience, that myosensory influences from the extrinsic muscles of the eyes play little or no role in stereoscopic acuity and so on. The existence of limiting disparities both in the transverse and in the vertical meridians is consistent with the known neuro-anatomic structure of the binocular visual system. The role of stereopsis among the empirical factors of spatial localization is clear.

On the basis of this evidence, one may adopt the point of view that stereopsis is a sensory phenomenon in its own right, with its own physiologic mechanisms.[11,42] Stereoscopic depth, while acting concomitantly with other visual functions, does not seem to be dependent on them. Fusion of the half-images is not a prerequisite for the emergence of stereoscopic depth, nor, conversely, does the existence of fusion imply stereopsis.

Stereopsis seems to be an all-or-none phenomenon, in that in a given person it is either present or not present. Training does not seem to develop stereopsis as such, but training may increase one's ability to discriminate depth differences, just as the visual acuity may be slightly improved by training. Ophthalmologic experience in the surgical treatment of strabismus seems also to bear out this idea that stereopsis either exists as a separate sensory response or it does not exist. Persons occasionally are found who have high visual acuity in both eyes, normal fusion and normal ocular movements and yet who do not show patent stereopsis.

This discussion is not to imply that all the problems of stereoscopic depth have been solved. However, it would seem that most of the remaining problems involve, in addition to the primitive response to disparity, the intervention of central processes. To mention a few: the problem of the magnitude of the subjective depth associated with observation distance, the figural "after-effects" in the third dimension of Köhler,[43] the compensatory reorientation of subjective space, when the eyes are in asymmetric convergence and as found in the induced effect, and the Pulfrich stereophenomenon.

Theories of Stereopsis

Although it is perhaps relatively easy to discover that disparity is the stimulus for stereoscopic vision, just how this disparity itself finally gives rise to the subjective experience is still a matter of speculation.

The earliest theories which regarded stereoscopic depth as being due to myosensory influences from the extra-ocular muscles in changes in convergence, or from the ciliary bodies in changes in accommodation, no longer can be entertained. At least, if these myosensory influences exist, they only aid the basic stereoscopic experience. The theory that stereopsis arises from the psychologic result of change in attention is not tenable, because the retinal stimuli that would provide the motive for change in attention already would have presupposed the depth.

The configurational theory that stereoscopic depth arises generally on the basis of differences in the Gestalt or in total forms of the images as perceived by the two eyes is unsatisfying in view of all the preceding discussion. This is not, however, to deny the possible importance of this aspect of spatial localization in one's understanding of stereoscopic vision.

A neurophysiologic theory can be assumed that interprets stereopsis as being a

specific sensory phenomenon arising from disparate stimuli within the neurologic structure of the occipital cortex, the origin of these stimuli being the dissimilarity in the optical images on the retinas of the two eyes that are transmitted by virtue of a stable organization of corresponding retinal points to that cortex. The unresolved aspect of this theory is whether this experience arises in its own right as a strict stimulus-response sensory process, or whether it arises by virtue of an intervening mechanism, the compulsion-for-fusion reflex. Stereopsis then would be a motor-sensory process.

Hering suggested that the binocular perception of depth, just as the localization of relative direction, depends on a type of local sign associated with the receptive elements of the retinas. Each retinal element situated transversely from the principal vertical meridian through the fovea, in addition to having a breadth directional value, also has a *depth value* in relation to its distance from the fovea. Elements on the nasal parts of each retina have "far" values. Similarly, retinal elements on the temporal parts of each retina have "near" values, these increasing the farther the elements are from the fovea. Hering assumed that these depth values provide an innate capacity for depth localization, although this aspect of the theory he considered to be of minor importance. The observer localizes related disparate images falling on the retinas of the two eyes "nearer" or "farther" than the apparent distance of the fixation point, according to the dominance (in the sense of an algebraic addition) of the "far" and "near" values from the two retinas. These local signs must be thought of as functional qualities of a special kind, in that *binocular simultaneity* is necessary and, furthermore, the disparity cannot be too great. In a way, one could speak, then, of an antagonistic effect of these depth values, and perhaps it is here where retinal rivalry plays a role. Attempts to find a depth experience from nasally or temporally stimulated elements of one eye *alone* have failed, and this failure has been cited to discredit the theory.

The theory of retinal depth values (a theory which Hering did not actually stress) is at present, as it has been in the past, not without opposition: first, because of a certain artificiality (*ex post facto*); second, because of an implication of innate spatial qualities to be associated with neuroanatomic structures; and third, because stereoscopic depth as a sensory experience can be suppressed in surroundings where conflicting empirical cues predominate.

This theory provides, however, a useful description of binocular perception of depth, one that is in agreement with actual facts for the eyes in *symmetric convergence*.

The view has been held generally that stereoscopic depth is somehow related to the processes of fusion in binocular vision. In fact, it is usual to speak of the presence of stereopsis as evidence of "third-degree fusion." The fact that stereoscopic depth exists with disparate images which are seen double has been taken by some as evidence that fusion is not associated with the emergence of stereopsis.

The theory that stereoscopic depth emerges through the fusion compulsion processes comes not only from general consideration but, specifically, from the well-known fact that disparate images of themselves alone can and do cause fusional movements of the eyes. These movements direct the eyes in such a way that the images are brought as nearly as possible to fall on corresponding retinal elements, thus eliminating the disparity and preventing the appearance of double images. This is true whether the disparate images are formed in the central or in the peripheral parts of the binocular visual field. In the normal use of the eyes, where there may be a pattern of different sets of transversely disparate images, these fusional movements cannot take place because *attention* is maintained at a point of fixation, the images of which are not normally disparate. Therefore, in the horizontal meridian one tends

to think of the fusional movements of the eyes that occur to avoid double images as being the result of *psycho-optical* reflexes. That is, once the attention is directed from one object of which the images are not disparate to one of which the images are disparate, the eye movements act as reflex movements. With the eyes directed to one object in space, the images of other objects in space, the images of which are horizontally disparate, provide constant innervations for fusional movements, movements which do not take place. It would be the central awareness of these innervations for fusional movements which would provide the basis for the stereoscopic depth, in that all innervations for eye movements would have sensory components. However, even this theory also rests on a notion of "local signs," this time motor values, which may or may not be identical with the local sign of relative direction. Such a theory would be consistent with the fact that stereoscopic responses may arise from disparate images which also are seen slightly double.

There are several bits of evidence, apart from theoretic considerations, that the motor-sensory theory is the less satisfactory. In the first place, measurements have shown that in the region near the point of fixation stereoscopic perception of depth ceases for disparities greater than about 20 minutes of arc (Fig. 3). Yet images with much greater disparities provide powerful stimuli for fusional movements. Second, the motor behavior patterns of the eyes in binocular vision are subject to re-education. This is evident from those patients who wear unequal ophthalmic corrections in the two eyes and who "learn" to compensate for the prismatic effect of the glasses, so that compensation becomes automatic with intended fixation eye movements. Stereoscopic perception of depth is not affected by such corrections unless the image magnifications on the retinas are at the same time affected unequally.

In the theory of stereoscopic perception of depth, while it is assumed that the depth is a specific sensory phenomenon related directly to disparity between the images in the two eyes, it is possible, if not probable, that the child learns to associate far and near depth to the basic sensation from image disparity, in the same sense that he associates the word "red" or the words "shades of red" with light stimuli of certain wavelengths, or with variations in those wavelengths. Certainly, the association of specific scales such as inches or feet to a basic stereoscopic response would have to be learned. This in no way detracts from the fundamental assumption that the stereoscopic sensation of depth is a unique sensory response to disparities between the images of separated objects in space.

In concluding this discussion, it is important to keep in mind the roles played by all the complex processes involved in our visual spatial localization, of which stereopsis is only one. The various factors and cues for this spatial localization interact with, complement, reinforce and perhaps inhibit, one another, depending on the varying visual surroundings of the moment and on the physiologic perfections of the visual apparatus. In one's daily work probably no one factor or group of factors dominates at all times. "Anyone who knows how pliable our spatial visual perceptions are under the influence of various conditions of observation and under the influence of past experience, taken into account consciously or unconsciously, should not be surprised at the multiplicity of results of observations on different objects and different observers" (Erggelet [40]).

It is not surprising that, if we select any one visual factor and attempt to find its importance in a subject's performing a given complex task, often no correlation or only a poor correlation is found. In particular, one can refer to the poor correlation obtaining between stereoscopic acuity as measured on a selected test apparatus in the laboratory and the flying ability of the aviator. This low correlation, and the equivocal associations reported in the litera-

ture, have been construed by some to mean that stereopsis is of little value to the pilot. This conclusion would seem unfortunate and unwarranted.

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DISCUSSION

DR. CRESCITELLI: "This is just for my information. What sort of data are available, if any, with regard to depth perception in animals, especially high-flying birds: hawks, eagles?"

DR. OGLE: "I do not know how one can really test for stereopsis in these birds. There is evidence, of course, that many of these birds particularly do have a little overlapping of their visual fields; and it has been assumed that, because of that overlapping, these animals have some stereoscopic depth. However, from great heights, it would not be very effective, since the intraocular separation is so small. True, the possible higher retinal resolution might tend to offset this factor. However, stereopsis might be very useful when the bird is within striking distance of its prey."

DR. BLACKWELL: "Dr. Ogle, would you wish to express any opinion as to why some people develop stereopsis whereas others do not?"

DR. OGLE: "I am only sorry that I do not know the answer to that question. I think there are not very many individuals who have good visual acuity and normal eye movements who do not also have stereoscopic depth. But, occasionally, they are found. It seems like, every year or so, some young resident intern in the Section of Radiology at the Clinic has trouble seeing the chest x-rays in stereo. I recall one in particular for whom all ophthalmic tests were normal. The visual acuity was high in both eyes, and fusion tests were normal. Still he never could see the chest in stereoscopic depth. I tested his stereoscopic depth with the Hering falling-bead test and, within a certain range, he could tell if the falling bead was farther or nearer than the fixation line. Thus if two objects were relatively near one another, it might be possible that he could tell which was the nearer; but he could not estimate how much. Thus the chest never appeared as a cavity in which details could be distinguished in depth. I wish I knew the answer to your question."

DR. BLACKWELL: "We have found the extent of binocular summation to vary considerably among normal observers. Could the development of stereopsis be correlated with the existence of binocular summation?"

DR. OGLE: "I am not sure, although I would doubt it. There have been some reports in the psychological literature to the effect that individuals who have the more rapid change of rivalry have higher stereoscopic ability. I do not know on what basis to explain this. One could, of course, relate it to the hypothesis which has been made by some psychologists that rivalry between stimulated disparate retinal elements is the basis for the emergence of stereoscopic depth."

DR. HERMAN M. BURIAN, University Hospital, Iowa City, Iowa: "This is difficult to understand. Isn't it true that rivalry relates to contours imaged in corresponding retinal areas?"

DR. OGLE: "Only upon the basis of the hypothesis just mentioned, and so far as I understand it, this is only an hypothesis."

Some Facts and Concepts Regarding the Neurophysiology of the Optic Pathway

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The outcome that is called vision is a product of a great many factors both broad and pervasive throughout the organism and peculiarly dependent upon specific tissues. No ordinary treatment can even touch on all of these factors. Some of them are possibly yet unknown, and a few of them, although known, have not yet been given much attention. It is this borderline of the known and the unknown and the little-dealt-with to which I am going to give some consideration in what I have to say. I shall discuss briefly four items: (1) the alternation of response theory; (2) brightness enhancement; (3) some factors underlying sensory fusion from intermittent stimulation; and (4) the nature of the distribution of photic radiation on the retina. Naturally, the four items are not mutually independent and certain of their interrelations will be brought out.

The first and third items have to do with the neurophysiology of the optic pathway; the second, with a form of sensory response; and the fourth item with how the distribution of photic stimulation of the retina relates to the message delivered the brain. All taken together, these items form some of the gross primary considerations involved in the operation of the optic pathway and of the person as a seeing organism. On this account, they seem to me to be quite pertinent in a discussion of visual mechanisms.

The Alteration of Response Theory

The first study of the neurophysiology of the optic pathway evolving kinds of information I wish to discuss was made by Bishop and Bartley, beginning in 1931. It is some of the findings and interpretations of the earliest of these that led to the alternation of response theory. This theory has arisen from the fact that no two repeated stimuli are very likely to find the central portion of the optic pathway in the same state. This became evidenced when it was found that repeated stimuli of fixed magnitude evoked various amplitudes of response in random order. This suggested some sort of possible periodicity in the activity of the pathway. That the brain is "spontaneously" and perpetually active is obvious and well known from records taken from the cortical surface from an unstimulated subject. The records consist, as is known, of a highly complex series of fluctuations of potential. Therefore, to suppose periodicity of function in specific pathways such as the optic was quite natural. Subsequent experimentation was devoted to ways of verifying the existence of and to disclosing the nature of optic pathway periodicity.

The first procedure was to attempt to tune repeated brief stimuli to the stump of the optic nerve so as to produce equal-sized cortical responses (Bishop[1]). This was done by varying both the intervals between stimuli and by shifting whole trains of stimuli back and forth. As a result, it was determined that the periodicity of the activity in the rabbit's optic cortex was 1/5 sec. According to where the rhythm was impinged upon, all large, all small, or all medium-sized responses were obtainable at will.

In another investigation (Bartley[2]) paired stimuli were used. The prime variable was the temporal separation between

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the members of the pair. Using maximally effective stimuli, it was found that two shocks could not be placed closer together than 1/5 second in the rabbit, if the second stimulus were to be as effective as the first. With smaller separations, the response to the second stimulus declined as the separation was diminished. If separations materially greater than 1/5 second were used, the response to the second stimulus first declined and then rose as separations were increased. At a separation of 2/5 second, a second response maximum was reached, beyond which a new decline set in. This, of course, was in line with Bishop's findings.

It was found that the results just mentioned did not hold if submaximal stimuli were used. The interpretation was that when maximal stimuli were used, all available channels were activated simultaneously and thus the optic nerve as a whole was acting as a single channel in its temporal aspects. Using maximal stimulation was one way to determine the essential properties of a single channel since a single channel could not be anatomically isolated. When submaximal stimuli were used, only a fraction of the total group of channels were activated by a single stimulus, and as a consequence the channels were definitely asynchronous in running their activity-rest cycles.

In using paired stimuli, the question of tuning into the intrinsic rhythm of cortex as in the very first-mentioned investigation, was not involved. The successive first members of the pairs of stimuli were presented quite far apart and therefore acted like isolated stimuli. The chances for all such stimuli were about equal for eliciting the same-sized responses. The technique of paired stimuli was also used with brief photic pulses as retinal stimuli and essentially the same principles were demonstrated as with electric shocks to the optic nerve.

A further investigation was made using trains of photic stimuli at rates greatly above the intrinsic rate of the cortical cycle. One of the main outcomes in this investigation was the following: when a train of stimuli at a rate from 15 to 20 per second was used, the first pulse in the train was, of course, responded to; the second elicited no response; the third elicited a submaximal response, and from then on for a few pulses varied results occurred. The response size ranged from zero to fairly good amplitude. Finally, this irregularity came to a definite end and all pulses elicited responses of the same size, but all were submaximal. This suggested that the available channels had become equally distributed between the members of the stimulus train. Bartley [2,3,4] called the initial phenomenon a reorganization period, and the result that followed, alternation of response.

The dependence of amplitude of responses on stimulus frequency was nicely demonstrated by Jasper and Cruikshank [5] in which they found that when photic pulse rate was 40 per second, the amplitude of cortical response was 1/4 of what it was at 10 per second, and when the stimulus rate was 20 per second, the response amplitude was 1/2 of what it was at 10 per second. As stimulus rates were slowly increased, transition (reorganization) states emerged, in which response waves underwent what they called desynchronization. It was during these transition periods that the amplitudes of the waves would drop to new and lower levels.

All of these findings very definitely suggest that the response results are determined by the ability of stimulus conditions to provide for a sorting out of the several channels in such a way that some channels respond to certain stimuli and other channels to others. Discussion of this matter brings into consideration the often-used term, *driving*. Driving has seldom, if ever, been given a strict operational definition. In general, when it can be observed that a series of response waves more or less follow the rate of the stimulus series, driving is imputed. The implication is, of course, that the usual activity-rest cycle in the cortical

elements has been altered by the stimulus to fit the repetitive pattern of the stimulus train. While this was, *a priori*, a possibility to entertain, it was not something to be taken as a fact on the kinds of evidence usually at hand. The evidence already cited (Bartley[3,4]) and (Jasper and Cruikshank[6]) would lead definitely away from the idea of the stimulus being able to change the intrinsic temporal value of the activity-rest cycles of the channels involved. Nevertheless, it may be appropriate to retain the term, *driving*, for certain results once it is made clear that the term refers to the regrouping of activity among the channels and not to altering the intrinsic character of the activity-rest cycles of the channels. Thus, driving is not to be thought of as so basic an effect as if the intrinsic activity-rest cycle of the channels were altered.

All of the foregoing and many other observations that have repeatedly been made throughout the last 25 years have led to the retention of the alternation of response theory that was first briefly mentioned by Bartley,[2,4] some years ago. It is appropriate that the theory be restated here. It will be observed that it is an intermingling of observation and deduction. It includes the following items: (1) There is a fixed number of parallel channels in the optic pathway. Although it is possible that even maximal stimuli do not always activate quite all of them, the recognition of a maximum is an appropriate starting point in envisaging the operation of the pathway. (2) Each channel possesses an intrinsic activity-rest cycle. While in the absolute sense these may not be of exactly the same magnitude, for certain beginning descriptions, they may be spoken of as the same. (3) These channels may be activated simultaneously or in many forms of distribution, the extreme being a temporally uniform pattern, in which as many channels are going into action as are going out at all instants. (4) Maximal isolated stimuli activate a near maximum number of channels simultaneously. (5) Maximal stimuli delivered at the rate represented by the intrinsic periodicity of the channels produce a train of responses, each representing a maximal synchronization of channels. (6) The closer to synchrony are the channels involved in the response, the greater its amplitude. Conversely, as temporal dispersion is produced for any reason, the amplitude drops accordingly. (7) This drop in amplitude, in the case of response to closely repeated stimuli, is produced by the channels becoming apportioned to various stimuli in the train. Each stimulus in the train is no longer able to activate all channels, owing to the arrival of the succeeding one before the recovery of the channels that have just been activated. (8) Continuous stimulation, after a very short period of reorganization, is finally responded to by a uniform number of channels being in their active phase at all instants. On this account, continuous stimulation elicits what amounts to continuous response rather than intermittent. This continuous response is of a lower amplitude than any over-all response that is intermittent. (9) Definitely submaximal stimuli do not succeed in activating at any instant as many channels as maximal stimuli. Hence by reason of leaving some channels unstimulated, a second submaximal stimulus is able to be effective sooner following a first stimulus than is the case with maximal stimuli. This may be because the activity of the first volley has changed the thresholds of the channels not yet activated or for some very different reason not yet hit upon. Nevertheless, we do know that the stated differences in the effects of the two magnitudes of stimuli do occur. There are certain known complications introduced at the lateral geniculate station that may account for the differences between the effective timing of maximal and submaximal stimuli. (10) As the temporal separation between paired maximal stimuli is lengthened from the point at which no response to the second is elicited, the result is graduated. That is, soon a small response is produced, and with increasing separa-

tions, larger and larger responses are produced until the response to the second is as large as the response to the first stimulus. (11) The same stimulus conditions that affect amplitude in the neurophysiological record of cortical response affect brightness in the sensory response. This statement is the linkage between the neurophysiological and the sensory (phenomenological) outcome. (12) It is assumed possible to check on the alternation of response theory as just outlined by using various temporal and intensity combinations of photic stimulation and by measuring the sensory outcome produced. One can set up a number of definite *a priori* expectations and test them. The theory can be extended into many corollaries, involving what to expect under various stimulus conditions.

Brightness Enhancement

Not long after the discovery of the major facts that led to the formulation of the alternation of response theory, examination of sensory responses to intermittent photic stimulation was undertaken, (Bartley[6,7,8,9] and Bartley and Wilkinson[10]). It was found that repetitive photic stimulation at some rates was more effective in producing brightness than was steady stimulation. It has long been known that at frequencies producing sensory fusion such stimulation is less effective than steady in producing brightness. Now it was found that as frequencies further and further below fusion were used, stimulus effectiveness began slowly and then more rapidly to rise until intermittent stimulation became equally effective. With still lower frequencies, it became even more effective than steady stimulation. In this case, the result was called *brightness enhancement*. A peak for brightness enhancement was reached, under some conditions, around 10 pulses per second. (See Figure 1.) It will be recalled that this is the region of the human alpha frequency. At this point, it should be mentioned that in our experimental animals the periodicity of the channels of the optic pathway and the alpha frequencies of the same animal were identical in value. In fact, the cells involved in the alpha activity and those involved in a portion of the cortical response were believed to be shown to be identical, (Bartley, O'Leary and Bishop[11,12]). Hence, when human brightness enhancement was discovered and found to be maximum with repetitive stimuli delivered at the alpha rate, it was taken to be a confirmation of expectations and of the idea that brightness is dependent upon

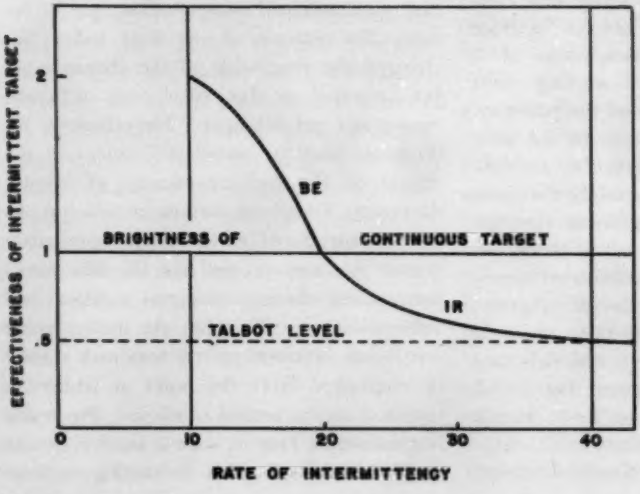

Fig. 1.—A diagram showing the number of photic pulses per second delivered the eye and their effectiveness relative to that of continuous stimulation of the same intensity. Section BE of the curve indicates brightness enhancement. Section IR is the range of intermediate effectiveness. The perpendicular at 40 per second indicates critical flicker frequency, or the rate at which for the particular stimulus train sensory fusion is produced.

central pathway activity as specified by the alternation of response theory.

The explanation of sensory fusion and the level of brightness produced by intermittent photic stimulation (in short, Talbot's law) has been conventionally accounted for by the photochemistry of the sense cells. The formulations of Hecht and others are too well known to require extended description here. With the demonstration of the existence of brightness enhancement, the question has arisen in some quarters (Jahn [13]) as to whether or not the same photochemical principles would account for it as well as the Talbot effect. Thus, we have two opposite factors to take into consideration: retinal photochemistry, and neural processes in the optic pathway. It is possible that to account for both the Talbot effect and brightness enhancement, it is necessary to take both factors into account, if the explanations are actually to follow the facts. We shall not address ourselves to the problem as it pertains to Talbot's law but will briefly outline the possible role of the two factors in brightness enhancement.

As in any case, one must consider the experimental operations used in the production or measurement of the phenomena in question. In measuring brightness enhancement, two visual targets are used. One is a steady field; the other an intermittent one, the intensity of which is adjusted so that the two will match in brightness for the observer. It is possible that there is a greater amount of light adaptation taking place in the steady target than in the intermittent one, while the two are under observation. To check on the influence of light adaptation in possibly changing the reference level of the steady target it may be possible to produce either a known different amount of adaptation in the two targets, or else to measure the loss in effectiveness of the steady target as a standard during the observation. Neither of the two possibilities has been fully tested, and thus some degree of brightness enhancement reported in the literature may be due to light adaptation in the steady standard target. While this may be true, it is certain that since brightness enhancement is dependent upon the peculiar range of frequencies of intermittent stimulation found to be effective, its existence is an expression of the activity of the neural mechanisms alluded to in the alternation of response theory rather than simply to something that we can call light adaptation.

A number of studies of brightness enhancement have been made. One of these (Bartley [7]) varied the intensity of the intermittent stimulus over a very wide range. As would be expected, brightness enhancement was not produced over the whole range. Only intense stimuli synchronize many channels in the optic pathway, even when the intermittency rate is in the optical range. This study also showed that at low intensities intermittent stimulation fell far below the conventionally expected Talbot effectiveness. With a pulse-to-cycle fraction of 1 to 1, at the very lowest intensities used, the intermittent stimulus was only $\frac{1}{14}$ as effective as the steady standard in producing brightness. This may have been due to the peculiarities of target arrangement, or it may have been due to the central mechanism described in alternation of response or both.

In another study (Bartley [9]), the effect of using the standard in one eye and the intermittent stimulus in the other eye was examined. Brightness enhancement was produced by this arrangement and the effect was greater than when using the stimuli in only a single eye. The interpretation rested on the elimination of incidental stray stimulation of the retinal area used for the intermittent stimulus. (See Distribution of Retinal Illumination.)

In still other studies, stray retinal illumination was held constant, (Bartley and Wilkinson [10]), and pulse-to-cycle fraction was manipulated (Bartley, Paczewitz and Valsi [14]). In this second study, shorter pulses, even though delivering less energy, should effect a better synchronization of

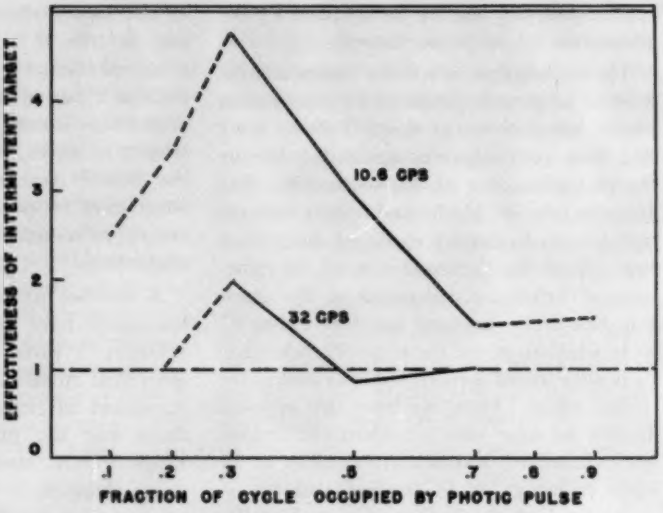

Fig. 2.—Graph to indicate the effectiveness of intermittent photic stimulation as a function of the fraction of the cycle occupied by the photic pulse. One curve shows the results with a pulse frequency of 32 cycles per second, and the other, with a frequency of 10.6 cycles per second.

channels in the optic pathway. On this account one should expect greater brightness from the shorter pulses. This was what was found. (See Figure 2.) Conflicting results were encountered with extremely short and extremely long pulses in the trains of fixed frequency. To explain them, it seems one must refer to what one would suppose would happen with and without off-responses being involved as conditions vary. The possible role of ganglion cell off-responses will be dealt with in the next section.

Sensory Fusion

The elicitation of flicker from sensory response has long been the chief matter studied in using intermittent photic stimulation. Some features of sensory response resulting from this sort of stimulation are well known, and others, just as important, are unknown or largely bypassed. Many years ago, Ferry[15] and Porter[16] each studied the relation between stimulus intensity and the rate of intermittency required to eliminate flicker. This rate has been

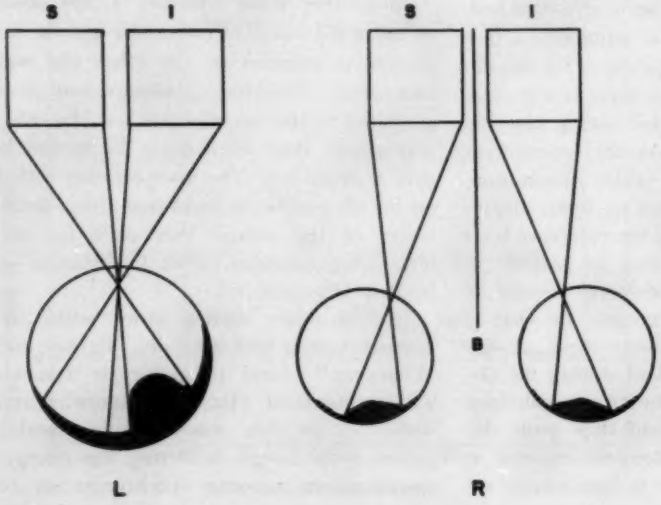

Fig. 3.—Schema L shows that the steady stimulus may cast some stray radiation on the portion of the retina meant to receive only intermittent stimulation and thus activate the retinal elements in the supposedly unstimulated portions of the intermittency cycle. Schema R is a diagram of an arrangement to avoid the unwanted results shown in L. B is a septum precluding radiation from opposite target reach the eye in question.

called critical flicker frequency (c. f. f.). The Ferry-Porter law states that the relation between critical flicker frequency and the logarithm of stimulus intensity is linear. This is true only for a certain midrange of intensities. It seems to be generally assumed that stimulus rate and flicker rate tally one-to-one. That this is not the case was pointed out and studied a number of years ago by Bartley.[17] Low intensities may require a critical flicker frequency of 10 cycles or less per second, whereas high intensities call for critical flicker frequencies in the 50's. Accordingly, the last vestige of flicker just before fusion is reached might be expected to vary anywhere between about 10 and 50 or more cycles per second. It turns out, however, that the last vestige of flicker has much the same frequency characteristics regardless of the pulse rate that is required to totally eliminate it. It would seem that the rate of the vestigial flicker lies in the region of 20 per second. Although this is counter to conventional expectations, it ought really to be no surprise, for there is a neuroretina that has to handle sense-cell input, and there is a central end of the optic pathway that has certain intrinsic properties which may become involved in whatever it does with the input it receives. If we grant then that the sensory result called flicker does not always tally in rate with the rate of the stimulus, we then have to consider how this discrepancy is produced. There are two known factors to consider. One is the fact that the sense cells are known to fire spontaneously at certain rhythmic rates characteristic of themselves (Granit[18]). This firing can be stopped or incidently induced by certain changes in the level of steady stimulation, etc. It might be that when stimulus repetition reaches a certain rate for a given intensity the effectiveness for producing flicker is lost, although these circumstances may be similar to those already found to underlie "spontaneous" sense-cell firing at rates intrinsic to the sense cells themselves. It may be, then, that the usually studied sensory fusion does not occur until this kind of firing is eliminated.

The second factor may possibly lie in the central end of the optic pathway. The behavior of the channels there has already been described. This behavior may play a determining role in the marginal outcome called vestigial flicker. Were this to be the case, vestigial flicker might be expected to possess the characteristics already found. Since the intrinsic periodicity of the channels in the pathway is somewhere near $\frac{1}{10}$ second, it would probably be true that no high rates of spontaneous flicker would eventuate. In fact, when better experimentation on this point is devised, it may be found that the frequency which Bartley took to be 20 per second, or thereabouts, will prove to be more closely 10 per second.

To say the least, the fact that flicker and stimulus repetition do not always tally in rate is a definite demonstration that the optic pathway is not the mere conducting system that it has long been tacitly implied to be, but plays a hand in determining the quantitative character of the sensory end result. In this connection, it is pertinent to point out that at various times in flicker experiments response has been observed to follow at half-rate instead of at full stimulus rate. This has been reported both by the Granit school of workers[19] and by Bartley.[3] B-waves in electroretinograms behaving the same way in repeated stimulation were also reported and would suggest that at times the retina rather than the central end of the pathway may be a prime determiner of flicker characteristics.

Not only has the electroretinogram manifested b-waves at half rate but when at full rate alternate large and small b-waves have been produced by successive stimuli. With an increase in stimulus rate, the small waves would drop out and responses would then follow at half the stimulus rate. While this sort of phenomenon bears on the question of what determines vestigial flicker, it also

is related to the kind of results described in the alternation of response theory.

One may suppose, hypothetically, that the off-discharge of the retinal ganglion cells plays a significant role in the perception of change in brightness such as is typified in seeing a light terminate or abruptly drop in brightness. We need experimental observation that will bear especially on the intimate features of the possible connection between the presence or absence of an off-response and the presence or absence of perceptual shifts just mentioned, and the conditions that underlie the phenomena. The following observations bear on this matter. Although the conclusions from them are highly interpretative rather than compellingly direct, they seem to be very plausible. Bartley [20] has shown that with nearly the same pulse frequencies very short and very long pulses can be used to just reach critical flicker frequency (c. f. f.). According to the very simplest expectations, if a series of short pulses at a given frequency just produce c. f. f., (uniform brightness), long pulses would exceed c. f. f. and part of the duration of each pulse in the series would be redundant. This is not the case. Therefore, according to the simplest view, the results are paradoxical. Bartley has used the ganglion cell off-response to account for the results. For example, it is known that the off-response may be inhibited in the electroretinogram if succeeding stimulation is presented quite soon after a stimulus that otherwise would produce an off-response. If it is assumed that the emergence of an off-response is the neurophysiological signal to the brain that a stimulus has terminated or abruptly dropped in intensity, then anything that would inhibit the emergence of the off-response would preclude the implied sensory end result.

Let us proceed further with interpretation. With a very short stimulus of a given intensity and a low enough rate of intermittency, flicker will ensue. If the same intensity and pulse rate are retained but the pulse length is increased, the pulse now occupies a greater fraction of the intermittency cycle. Whereas previously a train of pulses at the same rate did not succeed in producing uniform sensation owing to the extreme brevity of the pulses and the long intervals between them, uniformity in sensory end result is brought about by lengthening the pulses. Hence, we pass from flicker to fusion. With a further increase in pulse length, the pulses begin to be energetic enough to produce off-responses. Thus with a midrange of pulse lengths, flicker is brought back. Now, pulses can be lengthened still more until a point is reached at which the portion of each cycle that is left becomes so short that the off-response is inhibited by the arrival of the succeeding pulse. Thus the long pulse can be said to just reach c. f. f. or fusion just like the short pulse had done. Therefore with either a short or a very long pulse, one is able to just produce fusion, without radically altering pulse frequency, if at all.

The literature is well supplied with seemingly contradictory results from manipulating pulse-to-cycle fraction (commonly called light-dark ratio). Landis [21] has made a most extensive study of the diverse and contradictory results, and has not succeeded in finding a way to reconcile them. I believe that the presently given interpretation of the role of the off-response in the situation will reconcile the findings. I am suggesting that if the principles that I have just described were taken into account, the contradictions could be resolved, and predictions of certain other end results from the manipulation of the factors involved could be tested. In doing so, one must remember that the picture would have to take into account pulse intensity which, of course, will alter the length of pulse needed for the production of c. f. f., and thus change pulse-to-cycle fractions needed to produce the various end results in question.

Distribution of Retinal Illumination

The fourth item that deserves mention in describing some of the broad principles pertaining to the activity of the optic pathway

and resulting vision is the nature of the distribution of the photic stimulus on the retina. Just a few broad generalizations rather than a quantitative picture will be given. As far as visual targets are concerned, they may vary in the angle their images subtend on the retina, ranging from something quite small to covering the whole of the active retina. The simplest deductions from the ideal form of optics is that restricted targets activate only a portion of the retina and leave the remainder unlighted and thus unactivated. While it is possible to supply targets that will do this very thing, many or most of the restricted targets that are used in experimentation supply effective stimulation over a much greater portion of the retina than is represented by their images, and this makes a difference in the interpretations required in a great many kinds of experimentation.

The distribution that has just been alluded to accrues from the fact that not all of the photic radiation reaching the retina is focused in the image but that a material amount of it is widely scattered over the retina. This was first brought out in recent years by Bartley.[22] The matter was nicely confirmed by Boynton and Riggs,[25] and Boynton,[26] and Boynton, Bush and Enoch.[27] The only fact or principle that needs to be emphasized here is that in studying the relations between the gross quantitative patterns of retinal stimulation and various neurophysiological as well as sensory results, the effectiveness of stray photic radiation should be taken into account. This, for example, is particularly true in dealing with such matters as brightness enhancement. As will be recalled, two targets are used in its determination: one steady and one intermittent. If one were to suppose that the area of the retina supplied by the image of the intermittent target were going unstimulated during the inactive phase of the cycle of intermittency he would be making a mistake. (See Figure 3.) He would, in certain crucial cases, make the wrong deductions, not to say possibly set up his experiments in inappropriate ways to deal with some of the problems at hand.

Conclusions

The foregoing information with the interpretations just indicated seems to go a long way in accounting for certain of the major quantitative intensive and temporal features of sensory results in vision. They account for why brightness is greater for isolated brief stimuli, or for certain intermittent (repeated) stimuli, than for continual uniform stimulation. All such matters have been typically dealt with in terms solely of adaptation, a photochemical process in the retina. While this process is objective, it is not the sole determiner or the determiner at all of certain features of the sensory end result.

There is enough that is concrete and testable in the presented interpretations to make them worth further investigation.

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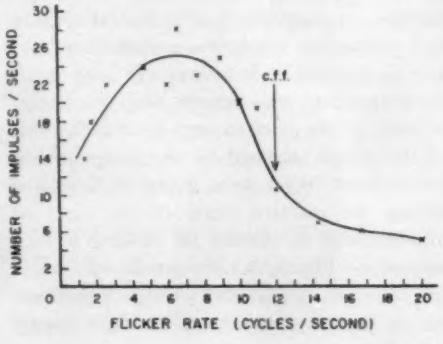
DISCUSSION

DR. DE VALOIS, Univ. of Michigan: "With regard to the problem of brightness enhancement, I would like to call attention to a recent paper by Grüsser and Creutzfeldt (Pflügers Archiv. f. d. ges. Physiol., 1957, 263:668-681) in which they offer a very nice explanation of brightness enhancement in terms of the frequency of spikes in cells in the optic pathway as a function of the duration of a light flash.

"What they find, recording the response of single cells in the cortex and retina to flickering light, is that the number of spikes per unit time increases to a maximum point as the flicker frequency is increased, and then falls again. The maximum number of spikes per unit time comes at 8 to 20 cps and is at a lower frequency than the fusion point, as is the case in the brightness enhancement curve. They show that this has no relation to the alpha rhythm at all—they have recorded the alpha rhythm simultaneously.

"We have confirmed these findings in recordings from single cells in the cat lateral geniculate nucleus. These results can be seen in slide one (Figure). It can be shown that this behavior of a cell to flickering light can be predicted from its response to a single flash. If one has a light on for a period of time, one finds a latent period with no response, then a rapid burst which tapers off in time. When one flickers a light at a very low frequency, one gets a response to each flash which includes not only the fast burst but also the low frequency later response. The total number of spikes per unit time is therefore some low value. When the flicker frequency is increased, one now gets just the rapid burst coming through to each flash and thus a higher number of spikes per second. If the flicker frequency is increased still more, one gets only a portion of the initial rapid burst to each flash and the number of spikes per second falls again; with a still faster flicker one finally reaches the fusion point when the cell no longer responds to each



flash. This curve corresponds exactly to the brightness enhancement curve, and the equivalence of number of spikes per unit time and brightness seems a reasonable one."

DR. LINDSLEY: "I would like to comment on that. In our own studies of brightness enhancement—three studies have been done—we have not found that brightness enhancement occurs solely in the alpha-frequency range. It does occur there; there is no doubt of that. But it extends down to five per second in one study done by Bartley's own method, or very similar to his method; and for some subjects two per second turned out to be the peak enhancement frequency. But, in some of our studies using two or three different methods, which, incidentally, were different in some respects from those Bartley had used, we found that brightness enhancement could occur in different subjects from five per second on up to twenty per second, with the predominant range being from about five to eleven, and more enhancement occurring in the range from five to eight than in the range from eight to twelve, which is the alpha-frequency range.

"But I do not see how this explanation which you have proposed (although I can see the difference between steady and flickering lights) would necessarily account for a selective region of flickering enhancement."

DR. DE VALOIS: "The rough correspondence of the enhancement maximum to the alpha frequency is apparently just a coincidence. The frequency at which you get maximum enhancement is a function of the way the cell fires to a single flash."

DR. LINDSLEY: "In terms of the frequency range for which process?"

DR. DE VALOIS: "The point they make is that the maximum point is a function of the rate at which the spikes occur. The higher the intensity of the light, the shorter the latency and, thus, the higher the frequency at which the maximum occurs. They get the peak anywhere from 8 to 20 cycles or so with an average of 10 cps."

DR. BLACKWELL: "It seems to me the important point here is that brightness enhancement can be 'explained' by the action of optic nerve fibers whereas previous explanation such as Bartley's, required cortical mechanism such as the alpha rhythm."

DR. DE VALOIS: "Grüsser and Creutzfeldt recorded this in the ganglion cells of the retina and also in the optic cortex."

DR. BLACKWELL: "And you have obtained the same result by recording from the lateral geniculate nucleus."

DR. LAWRENCE KRUGER, Johns Hopkins: "Since Dr. Marshall brought up the problem of the monkey, I would like to add that Dr. Charles Henry and I, several years ago studied the electrical activity in the visual cortex of about a dozen monkeys with chronically implanted electrodes, and were not struck by any relationship between the natural spontaneous rhythm of about 7 per second, and the excitability cycle.

"I also think a word of caution should be added concerning the relationship of the slow-wave evoked responses and the activity of individual neurons. It is fairly obvious by virtue of their different sensitivity to anesthesia that the slow waves do not necessarily relate to unit activity. This also is true of photic driving, in which the slow waves do not drive faster than about 70 or 80 per second, whereas single units can fire at substantially higher rates. One should emphasize that the slow phenomena we have been talking about (the evoked response) is very different from unit phenomena and that its characteristics cannot be translated into terms of the activity of neurons."

DR. BATTERSBY, Mt. Sinai Hospital, New York: "Just a brief comment. Some years ago, Dr. Krieger and I were doing some flicker work, and, quite by accident, had set our stimulator at about 8 or 10 cycles per second, as we started to calibrate test patch brightness with the Mac Beth illuminometer. When we pushed the switch down on the control box, thereby turning on the surround from the working standard of the illuminometer, we found that the brightness enhancement, which had previously been quite noticeable, completely disappeared. In fact, varying the relative 'on' to 'off' time in the cycle gave brightness measurements compatible with Talbot levels. We fooled around with this a little bit, and noticed that it was quite reliable; all observers appeared to get the same reaction. We never got back to this phenomena, but I was just thinking of a possible connection between our observations and Dr. Hartline's statement a few minutes ago concerning the lateral spread of inhibition in the eye of *Limulus*. It may be, for example, that our surround produced some lateral spread of inhibition in the retina which knocked down the brightness enhancement."

DR. H. K. HARTLINE: "Lateral inhibition exerted by a surround does alter the fundamental flicker response curve of single receptors in the eye of *Limulus*. The whole curve is moved up in the scale of intensity; also that the maximum frequency at which intermittency can be resolved is somewhat lower than when no surround is used. Inhibitory mechanisms perhaps do have an effect on flicker response even at very early levels in the optic pathway."

DR. RIGGS: "I do not have anything to add to what Dr. Hartline just said; I think it agrees with my recollection, too, of our old data.

"I just wanted to add one thing about the human eye; that is that, in the human electroretinogram, we too can find a building up to a maximum at certain frequencies. Very frequently, this occurs in a light-adapted eye, when you are using lights of different wavelengths, particularly at the red end of the spectrum, where the photopic responses are quite prominent.

"I will just give a single idea of what this might look like; with very low rates of flicker, the human electroretinogram clearly shows "on" effects and "off," let us say of this kind, where this would represent the response to a flash and this the response to the succeeding flash. If these are brought closer and closer together as you have with an increasing rate of flicker, it turns out that the superimposition of one wave on the other serves to give you a larger response. This may be because the "on"-response to a flash of light interacts with the "off"-response of another flash. The resulting responses to flicker of a moderate rate are large and sharply peaked.

"Since the ERG results primarily, we think, from the receptors and bipolars of the retina, we see that the basis for Bartley's enhancement of brightness with flicker may exist here also."

DR. LINDSLEY: "Following up on Dr. Bartley's comments about trying to relate neurophysiologic and psychological phenomena, I should like to show how we have tried to develop some parallel experiments in cats and monkeys and humans. The first slide (Figure 1) shows our set-up with the monkey in a Johnson stereotaxic instrument. There are electrodes on the cornea for the ERG, and electrodes in the optic nerve, optic tract, lateral geniculate bodies, superior colliculus and visual cortex. We record simultaneously from several of these regions during investigation of such parameters as intensity, wave-length, duration, frequency of repetition and so on. The next slide (Figure 2) shows responses to an increasing frequency of light flashes in the cat. Here it will be seen that subcortical stations follow the flash rate above 100 per second whereas the cortex seems to follow only to about 40 or 50 per second. This corresponds roughly with human CFF level.

"Now let me turn to another kind of correlation or parallelism between human and animal responses. If you present two relatively bright and very brief ten-microsecond flashes, 150 milliseconds apart, to human subjects, they will report two flashes; likewise at 100 milliseconds' separation. But, at fifty

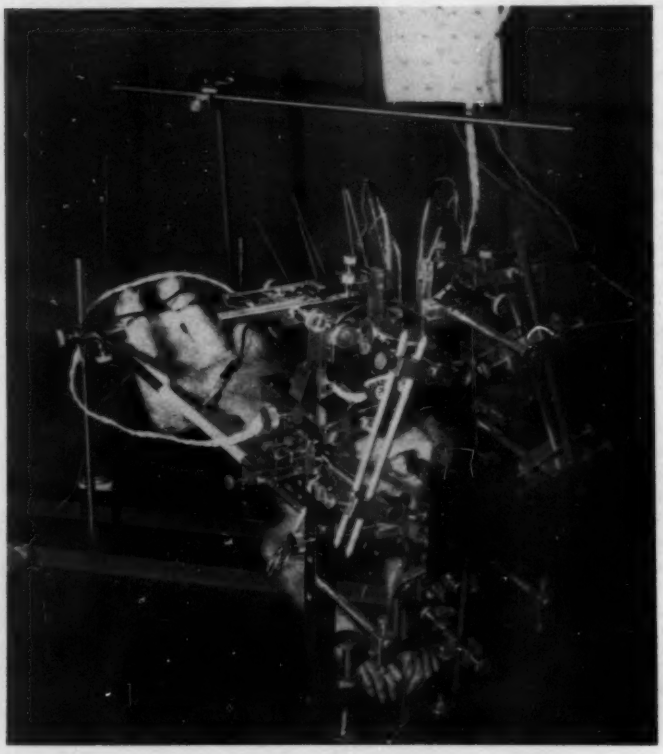


Fig. 1.—Monkey in Johnson stereotaxic instrument which guides electrodes to optic nerve, optic tract, superior colliculus, lateral geniculate bodies and optic radiations. Electrode holder (Grass) carries electrodes to cornea for ERG and to several locations on visual cortex. Electrical potentials recorded simultaneously from several stations along visual pathways at a time, during brief light flashes singly or repetitively. Light shield and tube restrict focussed beam of light to homatropinized pupil. (Lindsley and Griffiths, UCLA and VA Hospital, Long Beach, Calif.)

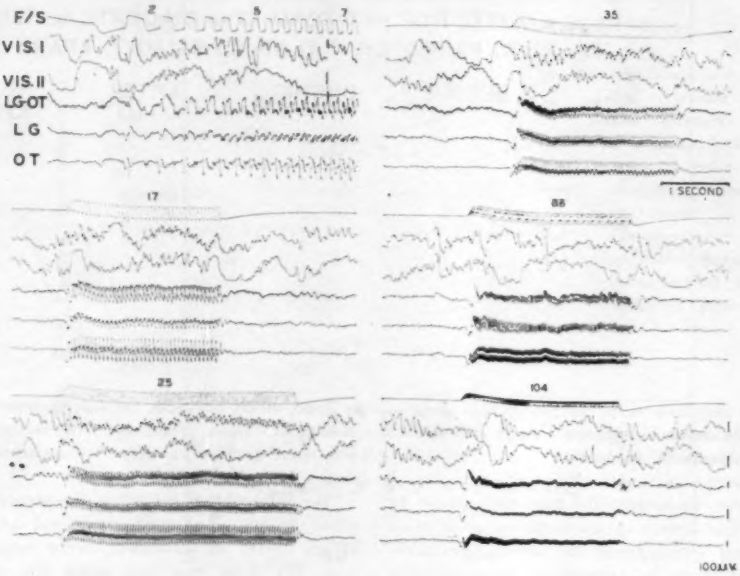


Fig. 2.—Repetitive light flashes of increasing frequency from 2 to 104 flashes per second. Electrical records from visual cortex (areas I and II), lateral geniculate (LG) and optic tract (OT). Area Visual I follows individual flashes at 35, but not 68 per second; subcortical stations follow each flash to well over 100 flashes per second. Records from cat: Unanesthetized brain (modified cerveau isolé preparation).

milliseconds' separation, they will report only one flash.

Figure 3 shows cortically evoked responses in the cat to a single flash (top record) and to paired flashes 50, 100, and 150 msec. apart in the lower records. Note that two distinct cortical responses (similar to the pattern produced by one flash) occur to pairs separated by 150 or 100 msec. but that only one results from a pair of flashes 50 msec. apart. Thus there is a correspondence, it seems, in terms of what the cat cortex will handle electrophysiologically and what the human psychophysical or subjective judgement will be to similar stimulus patterns.

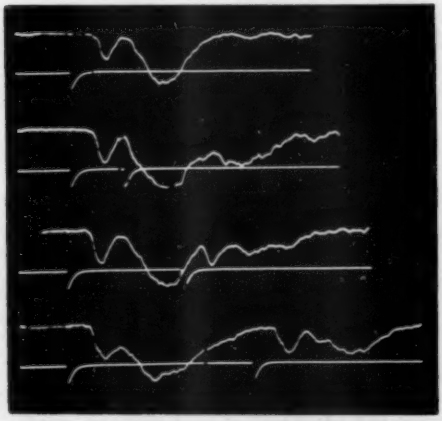


Fig. 3.—Response of cat's visual cortex to single, and to paired flashes of light of 50, 100 and 150 msec. apart. Note that two responses are obtained at 100 and 150 msec. separation, but only one evoked potential for the 50 msec. pair of flashes.

Figure 4 shows, in the cat, these pairs of 50-millisecond flashes producing one visual-cortical response when given at one-per-second rate. This occurred several times in succession. Then we stimulated the reticular formation for five minutes and for some ten minutes afterward we now got a double response in the visual cortex to these paired flashes which previously gave only one response. About 10-12 sec. after reticular stimulation the flash pairs evoked only a single response, like that originally produced. So here is a mechanism which seems to facilitate response in the cortex and suggests that the reticular formation aids in the finer temporal resolution of evoked potentials to paired light flashes.

"We have found some instances, however, when reticular formation stimulation could inhibit or suppress at least a portion of the geniculate response, particularly the second component; and we

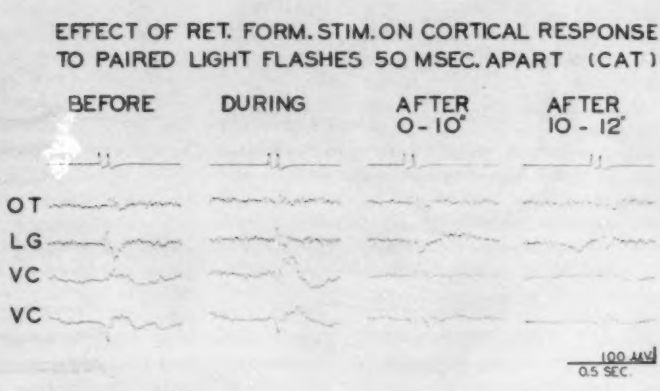


Fig. 4.—Cat visual cortex (VC) shown on bottom line responds with single evoked potential to 50 msec. pairs of light flashes before reticular formation stimulation, but gives two responses for 10 seconds following stimulation; thereafter returns to single response.

think this may also occur in the cortex. But, in the example shown in Figure 4 we certainly seemed to have evidence of facilitation.

In connection with Dr. Marshall's remarks, I would agree wholeheartedly that there must be excitability or activity cycles in the region of relay stations and especially in the lateral geniculate, but also no doubt in the primary receiving areas and in secondary cortex. We have not observed so far any modulation or fluctuation of the lateral geniculate response during repetitive stimulation; whereas this does seem to occur in the cortex, as Bishop indicated in 1933, and as we have observed also.

"One final thing in relation to what Bartley has said about timing relationships is illustrated in Figure 5. We presented an intensity of light which at twenty flashes per second produced fusion. These were successive flashes fifty milliseconds apart as shown in the top row. If we change the relationship of the same twenty flashes, so that the interval is 35-65, 40-60, or 45-55, the subject reports flicker instead of fusion. Then, as in the bottom row, if you come back to a fifty-fifty separation, it is fusion again.

"We think this is related to the phenomenon of photic driving or at least a temporal conditioning of the cortex to subsequent nerve response volleys. We think that only when this rhythm is maintained evenly is it possible then to get a succession of volleys through in the appropriate relationship. If the successive flashes and resulting nerve volleys do not fall in the appropriate excitable phases conditions by the rhythm they are perceived as flicker."

DR. ALPERN: "The classical investigators have worried about a problem which is quite relevant to this discussion. Broca and Sulzer among others asked the question 'How do the brightness of flashes or light of different durations (but of the same intensity) differ?' Although there is an extensive amount of literature on this problem the

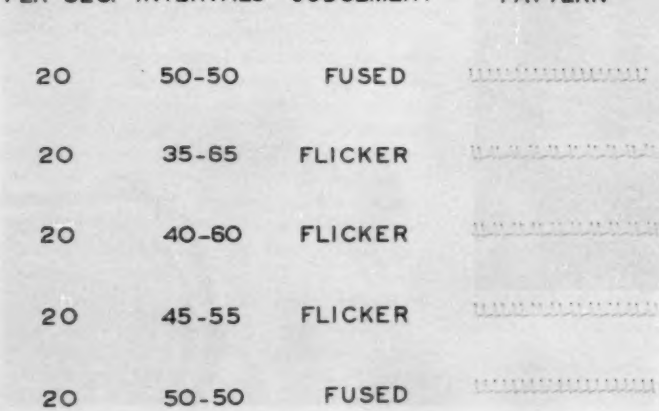


Fig. 5.—Flashes of light at 20 per second and evenly spaced cause fusion. Same light flashes distributed differently in time cause flicker in human subject.

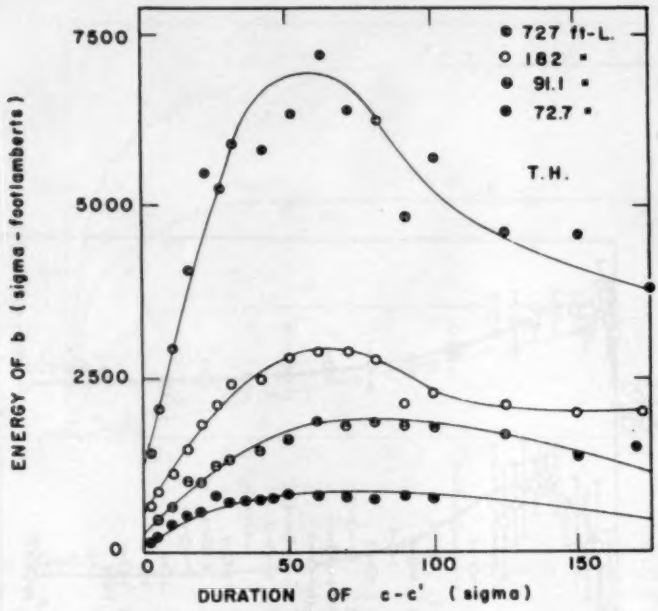


Figure 1

question is not easy to answer by experiments free of methodological criticisms. The first slide illustrates results when the measuring light was exposed to the left eye while the test flashes were exposed to the right. The detail procedure has been described elsewhere (M. Alpern, A Study of Some Aspects of Metacontrast, Ph.D. Dissertation, The Ohio State University, Columbus, Ohio, 1950) the ordinate shows the energy of the matching flashes required to match test flashes of differing durations and intensities. The abscissas are the durations of the various flashes. The important point is that for intense flashes of light a long flash appears dimmer than a shorter one. For certain durations there is a 'hyper maximal' brightness, i. e. a brightness enhancement over the brightness of the longer or shorter flashes. This is true only for the intense flashes, for less intense flashes it seems as though the longest flashes are the brightest ones of all.

One must be extremely careful in drawing analogies between these psychophysical data and electrophysiological data. Adrian (The Basis of Sensation, London, 1928) for example, tried to compare such curves with the response frequency of the discharge of the optic nerve as a function of time following a single long flash. The operational definitions of the abscissa values are so extremely different in the two cases that such an analogy strikes one as completely unreasonable. Nevertheless Adrian's figure persists even in Granit's most recent book (Receptors and Sensory Perception, Yale University Press, 1955, Fig. 137, page 282).

It is of course possible to draw valid analogies between the psychophysical data in the first slide and electrophysiological data, but surprisingly few such comparisons have been made. One attempt is by Dr. Faris and myself (J. opt. Soc. Am. 1956, 46:845-850. The next two slides have been reproduced from that publication with the permission of the Editor) is illustrated in the next slide. This shows the variation in the magnitude of the *b* wave of the human ERG for flashes of varying duration and intensity. Each curve shows the voltage fluctuations as a function of the durations of the flashes for a different intensity of the flash (the intensity values in trolands are designated on the curves). Long intense flashes produce smaller *b* waves than shorter ones but at lower intensities the largest *b* wave is produced by the longest flash. The next slide shows that a comparable effect does not occur for the *a* wave, even at the highest intensity studied.

"If, as I have suggested elsewhere (J. opt. Soc. Am. 1953, 43:648-657) the hypermaximal phase of the Broca and Sulzer curve (1st slide) is due to the fact that the tail end of the flash reduces the overall flash brightness as an example of metacontrast inhibition, then the fact that the *b* wave curves show a 'hypermaximal phase' but the *a* wave curves do not, has important physiological

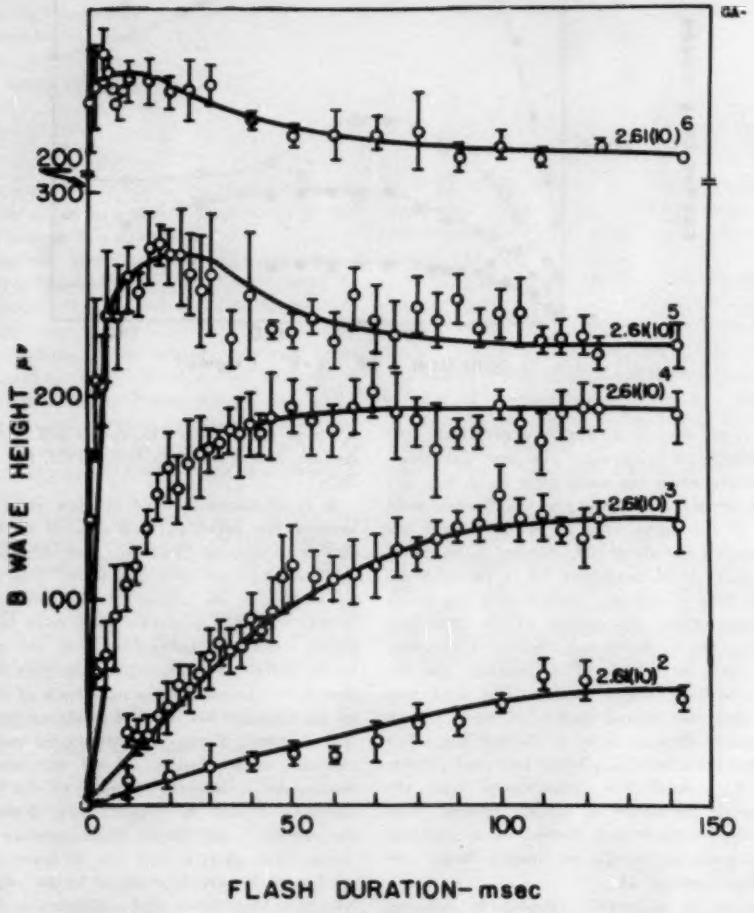

B WAVE HEIGHT μV
200
300
200
100
0
50
100
150
FLASH DURATION—msec
2.6(10)6
2.6(10)5
2.6(10)4
2.6(10)3
2.6(10)2

Figure 2

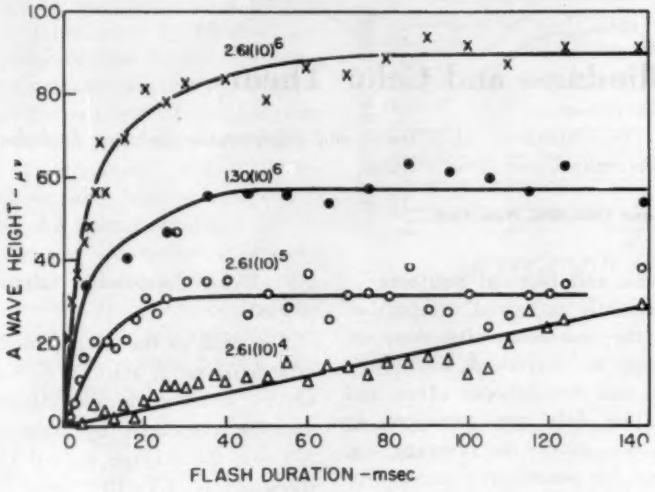


Figure 3

implications. It seems to me that the valid interrelation of Broca and Sulzer curves with other types of electrophysiological data can be an important way of gaining an understanding of brightness enhancement and other sensory phenomena as well."

DR. BLACKWELL: "I should like to point out to Dr. Walls that Bartley has provided an explanation for double-pulses arising from single photic stimulation and has shown doubleness in the response of the optic cortex under similar conditions to those in which the human observer experiences doubleness. Thus, I do not believe we have to look outside our present psychophysiological constructs for an explanation."

Color Blindness and Color Theory

Some Discriminations of Normal and Dichromatic Subjects Including a Unilaterally Color-Blind Person

C. H. GRAHAM and YUN HSIA, New York

Dichromatic and Normal Subjects

We have recently presented a report of research on the luminosity functions of normal subjects as contrasted with those of protanopes and deuteranopes (Hsia and Graham [8]). The data are presented in Figure 1 which gives the average log luminosity (or log sensitivity) curves for three groups of subjects consisting, respectively, of 7 normal subjects, 5 protanopes and 6 deuteranopes.[1] The basic data of the curves are relative energies required for the cones to respond to the spectral lights at the absolute threshold; the logarithms of the reciprocals of these values (i. e., log luminosity values) are here plotted.

The peak of the average normal curve is arbitrarily set at zero (i. e., maximum sensitivity is set at unity). Absolute energies may be calculated by observing that, at 578 mμ, the average normal (interpolated) threshold is 3.5×10^{-8} ergs. This figure amounts to about 10,000 quanta. Exposure duration was 4 msec. and the visual angle subtended by the circular stimulus was 42 minutes. These conditions fall within the limits of applicability of the Bunsen-Roscoe reciprocity law.

Columbia University.

This work was supported by a contract between the Office of Naval Research and Columbia University and by a grant-in-aid from the Higgins Fund of Columbia University. Reproduction in whole or in part is permitted for any purpose of the United States Government. The present manuscript reproduces considerable portions of previously prepared accounts.[4,6]

[1] The testing of our subjects involved the reading of the Ishihara and Stilling plates and the determination of the proportion of red and green required to match a yellow on the Hecht-Shlaer anomaloscope (1936). In addition, determinations were made, in the case of the dichromats, of the neutral point in the spectrum, that is, the wavelength that is seen as gray. The neutral point determinations were made with a modified Helmholtz colour-mixer.[7] The subjects included 15 men and 2 women (both of the latter having normal colour vision) between the ages of 20 and 35 years.

We are indebted to Professor Lucy J. Hayner of the Physics Department, Columbia University and Dr. Simon Shlaer, of the University of California, for technical discussions concerning our equipment.

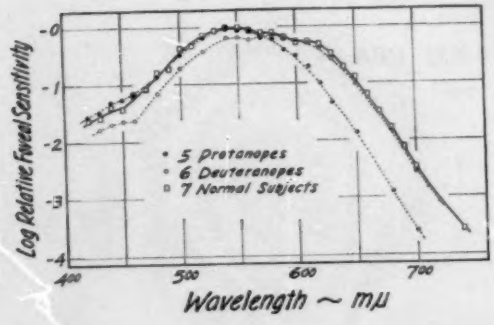


FIGURE 1. The luminosity curves for normal subjects, protanopes and deuteranopes in experiments by Hsia and Graham (1957).

The average log luminosity curve for five protanopes is represented by filled circles. It is to be observed that the average curve for protanopes shows a greatly decreased sensitivity in the red end of the spectrum as contrasted with the ones for normal subjects and deuteranopes. In the blue, the energy requirement for protanopes is similar to that for normal subjects.

The average log luminosity values for the deuteranopes is shown by the open circles. The luminosity for the deuteranope in the green and blue is less than the corresponding luminosity values for the normal subjects. In the red, the values are comparable.

The data of the normal curve are presented by crosses. The curve is a broad function that encompasses the extremes of the color-blind curves. As contrasted with the normal subject, the protanope has normal luminosity in the blue but shows loss in the red; the deuteranope shows normality in the red but a loss in the green.

Color Discriminations of a Subject Dichromatic in One Eye and Normal in the Other

The information obtained from the three groups of subjects has been greatly extended by our observations on a young woman who gave color-blind discriminations with her left eye and normal discriminations with her right eye (Graham and Hsia [3]). Her first test results seemed to indicate

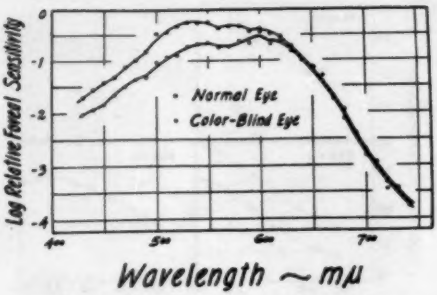


FIGURE 2. The luminosity curves for a unilaterally color-blind subject. The upper curve is the curve for the normal eye; the lower one, the curve for the color-blind eye. Data of Graham and Hsia (1958).

that she was deuteranopic, but as our observations multiplied, we found that our subject did not give completely typical deuteranopic responses.[2,3] In particular, as will be seen, her hue discrimination is better than that of the usual deuteranope in the blue and violet regions of the spectrum.[4]

Luminosity Curves [5]

The luminosity curves of our unilaterally color-blind subject are shown in Figure 2.

The curves for her two eyes show considerable luminosity differences in the blue and green regions of the spectrum. In this region luminosity for the color-blind eye shows a considerable loss below the level of the normal eye. It may also be seen that our subject's luminosity loss begins at wavelengths quite far into the red. In the red

[2] Dr. Gertrude Rand and Miss Catherine Rittler, both of the Laboratory of Ophthalmology, Columbia University College of Physicians and Surgeons, kindly examined our subject and concluded that, although her right eye is normal by all the tests used, including the Nagel anomaloscope, her left eye does not fit a simple category of classical colour defect. We deeply appreciate the cooperation of Dr. Rand and Miss Rittler in the testing of our subject. We are also indebted to Dr. R. L. Pfeiffer of the Ophthalmological Institute Columbia University College of Physicians and Surgeons, for the ophthalmological examination of our subject. No organic disease was found. A correction of −2 diopters is required for the right eye and −4 for the left. (During the experiment the correction lenses were installed just behind the artificial pupil, on the side away from the subject.)

[5] In addition to the tests (footnote 1) used on our other subjects we employed the following on our unilaterally dichromatic subject: Dvorine test (2nd edition), American Optical Company plates, Farnsworth Dichotomous D-15 and Farnsworth-Munsell 100-hue tests. Dr. Rand and Miss Rittler used the Hardy-Rand-Rittler plates as well as the two Farnsworth tests. They also used the American Optical Company and Ishihara tests as well as the Rabkin and Boström-Kugelberg tests. The Nagel anomaloscope was used on both eyes.

[4] For the historical background of work on unilaterally colour-blind subjects, see Judd.[9] Judd reports that 37 cases of unilateral colour blindness have been reported. Because of inadequate procedure or background information, only eight have proved useful for theory. The most recent research reported by Judd is that done by Sloan and Wollach.[12]

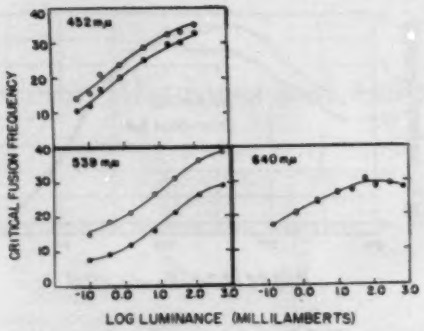


FIGURE 3. Critical fusion frequency as a function of luminance for wavelengths in three different regions of the spectrum. In each graph the open circles refer to data for the normal eye; the filled circles, to data for the color-blind eye. Data of Berger, Graham and Hsia.[1]

region of the spectrum, both eyes show similar luminosity values; little if any loss is evident.

Flicker Curves [1]

We have believed it important to find out whether or not the types of luminosity loss that occur at threshold for our subject are maintained at high intensity levels. In order to investigate this problem we, together with Dr. Eda Berger, have measured critical flicker thresholds at various intensities of different colors. The field of view was usually 28 minutes in diameter, but observations were also made with 1 degree and 2 degree fields.

A considerable number of curves in the normal and color-blind eye were obtained with a number of color filters, but the general nature of the result can be demonstrated by a comparison of curves for the blue, green and red regions of the spectrum. The data are shown in Figure 3.

The curves for blue light are shown at the top of the graph. In both the normal and color-blind eye, flicker fusion increases with intensity, the respective curves flattening out and then dropping at high luminance values. The positions of the curves differ, however. The curve for the color-blind eye is displaced downward on the flicker axis compared with the position of the curve for the normal eye.

The curves in the lower left-hand graph represent the data for green light. The displacements of the curve for the color-blind eye below the position of the curve for the normal eye is striking. In magnitude it is considerably more than was shown for the blue light.

In general, the fact of displacement means that, at any given intensity, fusion frequency is higher in the normal eye than in the color-blind eye in a manner reminiscent of the decrease in fusion frequency accompanying an increase in area. The latter statement may mean that, if fusion frequency represents available receptor units, then the type of color blindness represented by our subject is mainly attributable to the loss of green receptors and, possibly, but to a lesser extent, blue receptors also.

The story for red light is different: the same curve represents the data for the color-blind eye and the normal eye. No increased intensity requirement or fusion frequency loss for the color-blind eye is discernible in these data. No luminosity loss exists for red, even at the high intensities giving rise to fusion frequencies near the maximum.

Brightness Matches [1]

Another series of experiments bearing on the problem of luminosity loss at intensities above threshold was performed by us in collaboration with Dr. Berger. Specifically, we investigated the binocular brightness matches of our subject in various spectral regions.

The apparatus was so arranged that the subject could view two semicircles filled with light of the same wavelength. One semicircle was seen by one eye and the other by the other eye. They were viewed in such a way that they appeared to be side by side and close together.

The subject matched the semicircles in brightness by appropriate adjustments of intensity. The field of view for each eye subtended 1.8 degrees of visual angle. Eight spectral regions were investigated.

Measurements for any color extended over a luminance range of approximately 2.5 log units.

We shall not go into the details of the experiment. It is sufficient to say that at all levels of intensity, the intensity of a wavelength band centering on 538 mμ must, when it is seen in the color-blind eye, be greatly increased if it is to be judged equal in brightness to the corresponding light seen in the normal eye. A blue of 452 mμ requires a medium increase, and, in the red, the apparent brightness of the two colors are the same. Other colors show intermediate degrees of brightness loss.

The results of the present investigation point to the conclusion that the type of color blindness represented by our subject may be characterized as a loss or inactivation of part of the receptor mechanisms that mediate sensitivity in the green and (probably) blue portions of the spectrum. Furthermore, the data of binocular brightness matching and flicker show that the selective luminosity loss for our subject is not a phenomenon that exists only at cone threshold levels. It exists at all levels of intensity above cone threshold.

Color Mixture

Last summer, we had the opportunity, thanks to Commander Dean Farnsworth and Captain J. Vogel, of the Medical Research Laboratory, Submarine Base, New London, to examine the color-mixture functions of the two eyes of our subject. The color-mixture experiments were performed by Dr. H. G. Sperling and the data will be discussed in a later report which will be a collaborative endeavor.[5]

The curves in the upper graph of Figure 4 are the color-mixture data for our subject's normal eye. In these curves, a negative trichromatic coefficient means simply that a particular primary is combined with a test wavelength to match the two remaining primaries. The curves are to be contrasted with Wright's well known results [13,14] on ten normal eyes which are represented by the dashed lines. Our primaries are the same as Wright's: 460, 530 and 650 mμ. The units for the red and green primaries have been so specified that they have been taken equal at 582.5 mμ, while the green and blue primaries are the equal for the match 494 mμ. Our color-blind subject's normal eye gives, except for minor differences, the same sort of color-mixture data (including luminous units) as are represented by Wright's results. The color-mixture function for our subject's normal eye seems, in fact, to be normal.

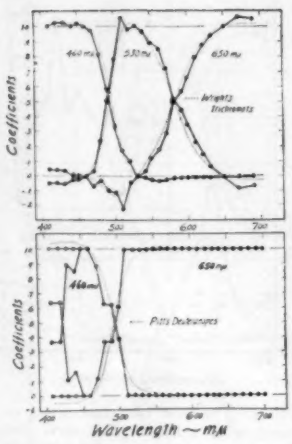


FIGURE 4. Data on color mixture.[5] The curves in the upper figure are the data for our subject's normal eye. The lower graph gives the results for the dichromatic eye.

The data for our subject's color-blind eye, shown at the bottom of the slide, are entirely different from the data of the normal eye. The graph shows that the subject can match any wavelength of the spectrum with a combination of two primaries, 460 mμ and 650 mμ (the units being taken equal at 494 mμ). The open and solid circles indicate two different types of results that were obtained in the short wavelength region depending on the method used. When the test wavelength was matched by the two primaries, the results

[5] A joint report from Columbia University and the Medical Research Laboratory under Bu Med Project Number NM 22 01 20 and under the authorship of C. H. Graham, H. G. Sperling, Y. Hsia and Anne H. Coulson.

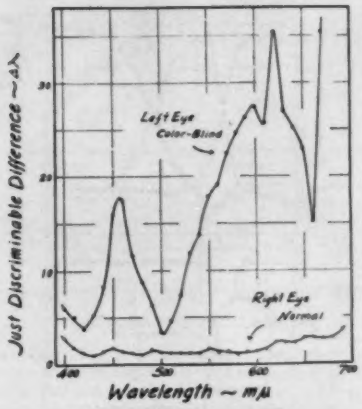


FIGURE 5. The hue discrimination of a unilaterally color-blind subject. The open circles refer to data for the normal eye; the filled circles, to data for the color-blind eye.

are as indicated by the solid circles. When the red primary was added to the test wavelength, the results are as given by the open circles. The latter method is the one used by Pitt [11] and it is seen that this result is much closer to Pitt's data, shown by the dashed lines, than are our other results. However, the first method gives results that seem to be more in line with our subject's data on hue discrimination.

Hue Discrimination

A hue discrimination curve was obtained on each eye of our subject. The curves are represented in Figure 5.

In general, one can say that the curve for the normal eye does not seem to be greatly different from the usual hue discrimination curve [14] obtained on normal individuals. The poorest discrimination, as shown by the largest $\Delta\lambda$, takes place in the red. Minima occur in the middle ranges of wavelengths. It is quite clear that the normal eye of our subject does not show defective hue discrimination.

The curve for the left eye, the color-blind eye, is an entirely different function. In the violet, the curve shows some insensitivity to wavelength change, but near 450 mμ it shows a rise in $\Delta\lambda$ indicating very poor hue discrimination. Discrimination improves to a minimum threshold value near 500 mμ in the region of the neutral point; thereafter it rises to high values near 600 mμ. The behavior of hue discrimination in the spectral region from 500 to 750 mμ seems to be of the sort found in deuteranopes.[11]

The hue discrimination shown in the short wavelengths may be in accord with our subject's color-mixture data obtained by matching a test wavelength by two primaries in her color-blind eye. The hue discrimination thresholds which show a minimum for the deuteranopic eye in the shortwave region may correspond to the steep slopes shown by the color-mixture curves in the violet near 450 mμ.

We shall finally consider some important theoretical data on color-naming and binocular color-matching.

Color Naming and Binocular Color Matches

The data on color-naming are clear. The subject calls all wavelengths below the neutral point (at about 502 mμ) blue. Above the neutral point, she calls all wavelengths yellow.

The experiment on binocular color-matching was performed by means of an apparatus, essentially a mirror stereoscope, that was so arranged as to provide slits of color in the left and right eyes. The sub-

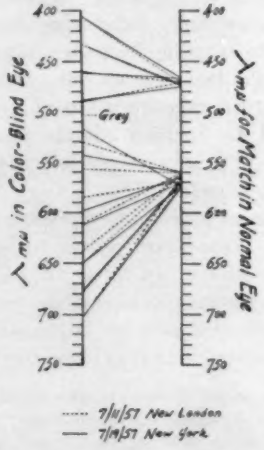


FIGURE 6. Results of the experiment on binocular color matching.[8]

ject, in viewing them, could see them side by side, the slit in the left eye being vertical, and the slit in the right eye being horizontal. The slits were so arranged that there was no apparent intersection of the images.

The results are summarized in Figure 6. In general it seems that, in her dichromatic eye, the subject matches all wavelengths greater than the neutral point (about 502 mμ) against a wavelength in the normal eye of about 570 mμ. In a word, all wavelengths greater than 502 mμ in the dichromatic eye are seen as a yellow of about 570 mμ in the normal eye. Wavelengths shorter than the neutral point in the dichromatic eye are matched in the normal eye by a blue of about 470 mμ. Thus the two sides of the spectrum below and above the neutral point are seen respectively as a blue equivalent to about 470 mμ and a yellow equivalent to about 570 mμ in the trichromatic eye.

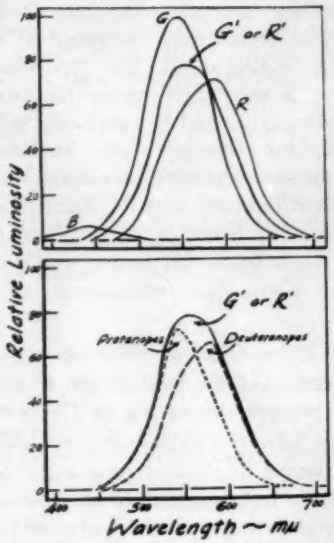


FIGURE 7. Theoretical curves. The upper graph represents the "transformations" R′ or G′ of the R and G curves to provide for the seeing of yellow by a dichromat who shows no luminosity loss. The lower graph represents the effect, for protanopes and deuteranopes, of luminosity losses.

Discussion

What can we say theoretically about our results?

First of all the fact of luminosity loss seems to be the usual finding in dichromats. The finding in the case of protanopes is pretty much as expected, and the finding for deuteranopes supports the results of Hecht and Hsia.[6] It should, however, be noted in this connection that one of our deuteranopes did not show a demonstrable loss. One may have to consider the possibility that, although the usual deuteranope shows a luminosity loss, not all do so.

In addition to the fact of luminosity loss our experiments demonstrate various important visual functions for the normal and dichromatic eye of our subject. We wish to emphasize one result, which we take to be, together with the fact of loss, a major outcome of the experiment. The important result centers on the color-naming and binocular color matching of our subject. It will be recalled that our subject's dichromatic eye sees wavelengths shorter than the neutral point as blue. How can the fact of dichromatic loss be brought into line with this result? How can yellow be seen for wavelengths above the neutral point by a dichromat who shows luminosity loss in the green if, as trichromatic theory demands, yellow is a mixture of green and red?

The account to be discussed is indicative of the type of thinking we have engaged in concerning the problem of dichromatic loss and the binocular matching of a unilateral dichromat. We shall here deal only with the seeing of yellow by dichromats.

Let it be supposed, as shown in the upper part of Figure 7, that in deuteranopia the red fundamental curve R moves toward the short wave part of the spectrum, to give the curve R′. The latter curve manifests a change in shape brought about, possibly, by virtue of the new absorption materials. At the same time, the green fundamental curve G moves towards the red and becomes G′. It is required that both curves become superimposed (or in constant ordinate ratio) in order to account for the fact that, as in

Leber-Fick theory,[2,10] they provide a sensation of yellow over the range of wavelengths embraced by the "transformed" curves. A wavelength which stimulates the now identical G′ and R′ substances will give yellow, for although both fundamentals have the same absorption spectrum, they are connected centrally with the usual R and G mechanisms. Some of the implications of these suppositions may be analyzed in terms of the König-type fundamentals of Figure 7.

For a condition of color-blindness, the luminosities of the two similar R′ and G′ curves (upper part of Figure 7) must sum to give normal luminosities. In addition, the ordinates of constant ratio in the curves R′ and G′ represent a dichromat for whom a central mixture of the identical fundamentals causes a seeing of yellow throughout the longwave region of the spectrum. Such a dichromat would show no luminosity loss. We have mentioned the fact that one of our deuteranopes could be so described.

Any luminosity loss can be introduced by assuming a curve for deuteranopes or protanopes that lies beneath the R′ or G′ curve as in the lower graph. The respective curves here were plotted in terms of the ratios of (a) protanopic average luminosity to normal and (b) deuteranopic average luminosity to normal (based on our group data of Figure 1). Since both curves fall below the R′ or G′ curve, the luminosity values will show a loss below the sum of R′ and G′ that is equivalent to normal luminosity.

The account as presented may be considered for what it is worth. Its merit may lie in the extent to which it approximates a useful statement of how trichromatic theory can account both for luminosity losses and the seeing of spectral colors by deuteranopes and protanopes.[6]

[6] We are greatly indebted to our subject for the many hours that she devoted to observing in and discussing our experiments.

Summary

1. Data have been obtained on three groups of subjects, made up, respectively, of 5 protanopes, 6 deuteranopes and 7 normal subjects. As compared with normal subjects, protanopes show a loss of luminosity in the red. This is the usual finding. In conformity with the results of Hecht and Hsia (1947), deuteranopes are found to show a loss of luminosity in the green and blue.

2. Experiments have been performed on a young woman whose right eye exhibits normal color vision and whose left eye is dichromatic.

3. This subject's luminosity function shows a loss of luminosity for the dichromatic eye in the green and blue regions of the spectrum.

4. As shown by data on flicker and brightness matches, the loss of luminosity is maintained at relatively high intensities of illumination.

5. The normal eye of our subject gives color mixture curves that are similar to those given by the usual trichromats. In her dichromatic eye, she can match any wavelength in the spectrum with a combination of two primaries, 460 mμ and 650 mμ.

6. The normal eye of our subject does not show defective hue discrimination. Hue discrimination thresholds in her color-blind eye are raised above their values in the normal eye. However, in the violet below 440 mμ, her hue discrimination is better than it is in the case of the usual deuteranope.

7. Our subject named the colors that she saw with her dichromatic eye in terms of colors she saw in her normal eye. Wavelengths below the neutral point (which occurs at about 502 mμ) in her color-blind eye are called blue; above the neutral point they are called yellow. At and near the neutral point they are called grey.

8. Data on binocular color-matching support the results of color naming. Our subject's dichromatic eye sees only two colors in the spectrum: a blue equivalent

to 470 mμ (viewed in her normal eye) for wavelengths below the neutral point, and a yellow equivalent to 570 mμ, for wavelengths above the neutral point.

9. The seeing of yellow by deuteranopes and protanopes may be accounted for by an idea based on Fick-Leber theory. It is assumed that the characteristic sensitivities of the red and green receptors become similar while no change takes place in their central brain connection. Losses may be introduced into the changed sensitivity curves to specify luminosity deficits.

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DISCUSSION

DR. LOUISE SLOAN (Wilmer Institute): "If the color blind eye sees only blue and yellow you would expect that the wavelengths which look alike to the two eyes would be those which the normal eye saw respectively as pure blue (i. e. not greenish or a reddish blue) and pure yellow. The yellow chosen by my subject as looking alike to the two eyes was to most normal eyes a *reddish* yellow. Judd thought that this discrepancy could perhaps be attributed to an abnormal macular pigmentation. I notice that your subject picks as the regions that look alike to the two eyes, 470 and 560. These again do not correspond exactly to the spectral locations of unitary blue and yellow."

DR. GRAHAM: "Any blue in our subject's dichromatic eye should match 470 mμ as seen in the normal eye."

DR. SLOAN: "When you speak of hue discrimination it is really wavelength discrimination which subjectively depends upon differences in saturation of the same hue."

DR. GRAHAM: "Yes. The dichromatic eye is probably discriminating on the basis of saturation."

DR. BLACKWELL: "I might remark that the notion that deuteranopia can be a loss-system receives support from the discovery of blue monocone monochromats which my wife and I reported last March before the Optical Society. We found four cases of total color blindness at photopic luminance levels, in which the luminosity curve was extremely narrow, with a peak at 450 mμ. Pitt has argued that monochromacy could be produced by double dichromacy. If this were the case, the blue mono-cone monochromats could only be produced by double dichromacy if deuteranopia were a loss-system. It is interesting that the mother of three male blue mono-cone monochromats had normal color vision and acuity, but was found to possess a few foveal rods. Perhaps this is the carrier sign of a new color vision defect."

On-Responses in the Human Visual System as Inferred from Psychophysical Studies of Rapid-Adaptation

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At the University of Rochester in the past few years, we have been doing work with Public Health support along two entirely different lines. One part has involved the measurement of scattered light and retinal image quality, involving work with excised steer eyes and occasionally other animals. The other work has involved an investigation of the transition from the absolute cone threshold, on the one hand, to the luminance-discrimination threshold on the other. It is the second part of the work that what I wish to say today is based upon.

I would like to acknowledge the help of Dr. William Bush, Dr. Jay Enoch, Mr. Gillray Kandel, and Mrs. Judith Onley, who have participated in various of the experiments which we have done with respect to this particular problem.

I first became interested in this area as a result of reading two articles and seeing a connection between them. One of these was published in 1949 by Howard Baker,[1] who measured the time course of light-adaptation in a new way. The procedure was simply to turn on a large adapting stimulus, and measure the intensity of a flash of light required, at various times after adapting stimulus onset, for threshold visibility. He was surprised to find that the threshold of the flash, instead of being progressively elevated by exposure to the adapting stimulus, decreased for a time instead.

The other study was done in England during the war by B. H. Crawford.[2] He presented his observations with a repetitive sequence of "conditioning" stimuli, (each lasting 0.52 seconds) one about every seven seconds, and measured the threshold to test flashes delivered at various times during the sequence. Thus, the test flash was sometimes delivered during the dark interval between conditioning flashes, sometimes during the light interval while a conditioning flash was present, and sometimes just at the time the conditioning stimulus came on or went off.

During the time that the conditioning stimulus was present, his experiment was quite similar to Baker's except for the time intervals involved. Whereas Baker's first measurement was taken after about 15 seconds in the light, Crawford's measurements were all taken within the first half-second. And during this half-second period, there was clear evidence of the same kind of threshold decline that Baker noticed over the range from approximately 15 seconds to three minutes.

Crawford found that the amount of threshold rise that could occur, almost instantaneously, around the time of conditioning stimulus onset, was perfectly enormous. An increase of ten-thousandfold can occur in the space of 100 milliseconds or less. At the peak of the effect, the luminance discrimination threshold, $\Delta B/B$, reaches a level which is about 50 times what it will be when steady-state adaptation is reached.

We interpret the initial rise in threshold associated with the onset of the conditioning

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stimulus as follows: When a conditioning stimulus is delivered to the dark-adapted eye, the visual system is momentarily thrown into a relatively refractory state, where only an unusually intense test flash is capable of adding a discriminable increment to the burst of activity produced by the onset of the conditioning stimulus. In other words, the visual system is so busy responding to the onset of the conditioning stimulus that it becomes unusually difficult to "push through" additional information pertaining to the test stimulus.

This position is very similar to the one that Baker has taken, as indicated by the following quotation from one of his articles:[4]

"When the adapting field is turned on, the dark-adapted receptor in the eye begins responding almost at the maximum rate possible, and it takes a disproportionately greater ΔI at this time to cause the receptor to increase its response. Later, as the discharge of the receptor slows, less and less additional light is needed to make the receptor respond noticeably more, until finally the ΔI is limited only by the photochemical balance in the receptor. After that time, at about three minutes, residual photochemical bleaching causes the ΔI to drift upward." (Baker has mentioned the fact that Graham and Riggs, many years ago, working with the *Limulus* eye, found the same kind of effect, using a criterion frequency increment of the single optic-nerve discharge as a criterion. They were rather puzzled about it, too, at the time they discovered it.)

To summarize the ideas just presented then, allow me to restate the situation as follows: When the previously dark-adapted eye is suddenly presented with an adapting stimulus, a photochemical reaction begins in each of the receptors involved. Associated with this very high level of photochemical availability, and other factors which are none too well understood, is the instigation of a very high level of activity in the neurons leading to the higher visual centers. This, of course, has been observed many, many times in many different kinds of physiological preparations, most elegantly, I think, in Dr. Hartline's single *Limulus*-fiber preparation. As a consequence, it may be very difficult to perceive a test stimulus superimposed upon the onset of the adapting stimulus—not because the sensitivity of the eye is low for photochemical reasons, but more especially because of the already ongoing activity. In other words, the perception of the test stimulus is *masked* by the onset of the adapting stimulus. As the eye becomes progressively more light-adapted, (as the conditioning stimulus remains on longer and longer) the sensitivity of each receptor involved becomes less, as indicated by classical photochemical theory. Meanwhile, however, the response rate in the visual nerve fibers is also subsiding. Thus, we have one factor (*activity rate*) which is working in the direction of increasing sensitivity to the test flash, and another (*photochemical depletion*) which is working in the opposite direction. Consequently, it is possible for the threshold to drop during this initial period, despite the progressive decrease in receptor sensitivity. After a time, the photochemical steady-state is reached. Some of Baker's [1,4] data indicate that the threshold will rise again before this time, whereas others of his data indicate that it will not. Which of these happens depends upon the balance of these two factors, which I have referred to as "photochemical depletion," on the one hand, and "masking" on the other.

That masking may occur before an appreciable photochemical depletion has set in is further suggested by the fact that significant threshold rises can be observed when the test stimulus slightly precedes the adapting stimulus. In such a case, the level of photochemical availability is clearly as high at the time the test flash is delivered as it possibly can be. The eye is completely dark adapted.

Why should this happen? I do not think that it is probably too mysterious. The activity engendered by the test flash, which

is dimmer and smaller than the conditioning stimulus, takes longer to be transmitted through the visual pathways than that resulting from the brighter, larger adapting stimulus. Consequently, a seemingly retroactive masking effect takes place. Just where it takes place in the visual system is uncertain. Some of it may be at the receptor level (we got some evidence[7] some years ago to show that you could get this sort of thing using an ERG as a response measure). Part of it, however, must occur well beyond the receptor level, because it is possible to get some masking via stimulation to the contralateral eye—not a great deal, but nevertheless a significant amount.[8]

The main thing that I wish to do now is to describe an experiment which was designed to throw additional light on these matters. Although it is an experiment which has already been published,[9] I would like to describe it again now because I have a feeling that it may be an important one; and, at the very least, it is the reference experiment to which the rest of our work along these lines should be related. I will begin by reading the list of conclusions that came out of the experiment:

(1) The conditioning stimulus-test flash technique is capable of producing an indirect record of "on-responses" in the human visual system. If one works with the dark-adapted eye, the raw data provide such a picture; if one is dealing with the light-adapted eye, the photochemical effects can be factored out.

(2) Under conditions where the prevailing level of receptor illumination is rapidly changing (which includes almost all normal vision), the sensitivity of the human eye is by no means determined solely by photochemical factors.

(3) Under some extreme conditions, threshold is determined solely by the masking effects produced by on-responses somewhere in the visual system which block the transmission of information that otherwise would signal the presence of the test stimulus.

(4) Under most conditions, both photochemical factors and masking operate together to determine the threshold.

(5) Under some conditions, increasing the adaptation level of the eye can result in a *decrease* in threshold.

I would like your particular attention to the final conclusion:

(6) Under steady-state conditions, masking is so slight that photochemical factors alone determine the threshold in the same way that they do in the otherwise unstimulated eye.

That this has not been the prevailing view is indicated by the elaborate dodges that experimenters have used, in studies of adaptation, to *remove* the adapting stimulus from the part of the visual field to which the test flash is to be delivered. MacAdam,[10] for example, in investigating chromatic adaptation, uses a stimulus cycles in which the adapting stimulus, which is present most of the time, is *suddenly replaced* by the test stimulus while a match is being made with a third stimulus delivered to the other eye. Blanchard,[12] many years ago, studied the so-called "instantaneous threshold," and also used a technique where the adapting stimulus was suddenly cut off. Some studies of luminance discrimination (for example, those of Holway and Hurvich[11]) also involved a temporal sequence of some sort. Many other studies have used a surround illumination but, care is taken that the surround does not include the test area. Thus, into a black hole in the middle of the adapting field, the test flash is thrust. Now if my conclusion is correct I woud have this to say about each of these procedures: (1) The sudden replacement of the adapting stimulus by the test catches the eye in a dynamic state of off- and on-activity in which the experimenter is generally not really interested and which greatly complicates his interpretation. As far as luminance discrimination is concerned, one can, I'm sure, get lower and more stable values of $\Delta B/B$ simply by superimposing the test flash upon the adapt-

ing field. (2) As far as the surround is concerned, it is used in various experiments mainly because it may have a considerable effect; otherwise, people would not bother with it. My own view [13-15] is that its effect is usually the result of stray light cast across the retinal area which receives the test stimulus. For the types of surround generally used, it would be a most difficult job to calculate this level, though it could be found empirically by using a full-field stimulus which produced the same result. If the surround comes very close to the test area, there are doubtless retinal inhibition effects at work. (The direct experimental evidence for inhibition of this kind in the visual system has nowhere been more brilliantly demonstrated, I think, than in the type of work Dr. Hartline reported yesterday. It has not been too long since this sort of thing was pure speculation.) Unless the experimenter is particularly interested in these, I think he would be better off to avoid them.

In other words: If you wish to change the state of adaptation of the eye, simply fill the visual field with light and allow the subject to be exposed to it for 5 or 10 minutes. Then, do whatever experiment you wish, simply by superimposing your stimulus conditions upon this adapting field. Throw away your fancy timers and your black holes—they're not really needed.

Now I will attempt to describe to you the experiment which I believe supports these contentions, as well as the other conclusions that I quoted a moment ago.

Fig. 1 shows the stimulus conditions of the experiment. The pre-adapting stimulus is the largest of the three circles you see here. These stimuli were seldom simultaneously presented to the subjects during the experiment but, when they were, this is what they looked like. The adapting stimulus subtended 13°; the conditioning stimulus, which is the kind of short stimulus to which I was referring in connection with Crawford's experiment, is slightly inside of that (11° of visual angle).

The test flash is foveal; so we are dealing with cone responses, one degree in diameter, and the fixation arrangement is such that we can always be sure that the test flash is being delivered to the center of the fovea. So we have three stimuli: the pre-adapting, the conditioning, and the test. That is all there is to it.

The next figure (Fig. 2) shows the temporal stimulus sequence. This is perhaps a little bit harder to keep in mind. For the main experiment, there are two major conditions indicated here by "on" and "off." The words "on" and "off" refer to the presence or absence, in the two cases, of the conditioning stimulus. Otherwise, the sequence is the same in each case. I will describe the stimulus sequence for the so-called "on" condition first.

The subject has been sitting in the experimental room and has been adapted to some pre-adapting level. It may be zero; or it may be over 1000 mL. It depends on the condition. (Conditioning-stimulus luminance, however, is *always* 38 mL.) He

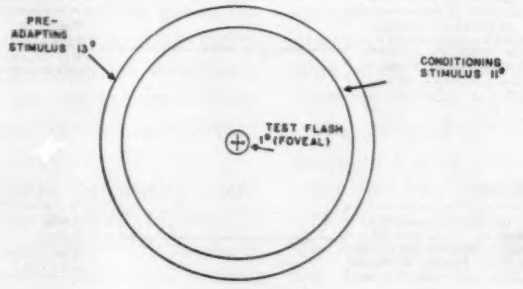

Figure 1

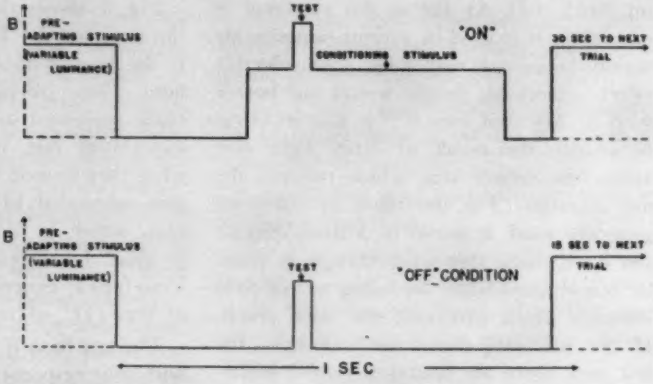

Figure 2

has seen it long enough so that we can be fairly certain he is adapted at a steady-state level. Then, after a warning signal, the pre-adapting stimulus suddenly disappears. It is dark for slightly less than three-tenths of a second; this is followed by the onset of the conditioning stimulus which, in turn, (in this example) is followed by the onset of the test flash. The test flash lasts about a thirtieth of a second, then goes off; the conditioning stimulus remains on altogether for about a half-second; then it goes off. Then, very quickly thereafter, the pre-adapting stimulus reappears.

Therefore, the eye actually is disturbed from its original level of pre-adaptation only for a very short time, on the order of three-quarters of a second; then sufficient time is allowed for the subject to return to his previous state of adaptation.

These thresholds actually are obtained by using one time-interval setting at a time, and by repeating the sequence over and over again, and using a descending method of limits. The subject is asked to pay attention only to the test flash. This may sound difficult; but, actually, it is a judgment which proves to be not particularly difficult at all. For the "off" condition, we have the same situation, except that the conditioning stimulus is not present. For reasons which will become evident in a moment, the time setting for the test flash in the "off" condition will be referred to the point where the conditioning stimulus would have come on, had there been one. The time interval between the onset of the conditioning stim-

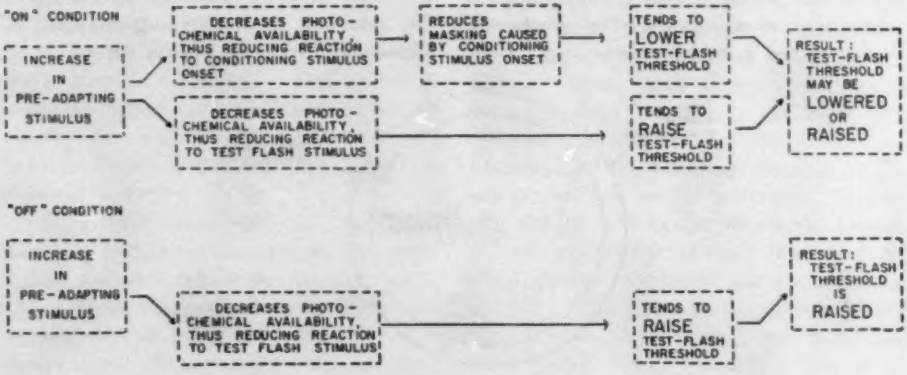
"ON" CONDITION
INCREASE IN PRE-ADAPTING STIMULUS
DECREASES PHOTO-CHEMICAL AVAILABILITY, THUS REDUCING REACTION TO CONDITIONING STIMULUS ONSET
REDUCES MASKING CAUSED BY CONDITIONING STIMULUS ONSET
TENDS TO LOWER TEST-FLASH THRESHOLD
DECREASES PHOTO-CHEMICAL AVAILABILITY, THUS REDUCING REACTION TO TEST FLASH STIMULUS
TENDS TO RAISE TEST-FLASH THRESHOLD
RESULT: TEST-FLASH THRESHOLD MAY BE LOWERED OR RAISED
"OFF" CONDITION
INCREASE IN PRE-ADAPTING STIMULUS
DECREASES PHOTO-CHEMICAL AVAILABILITY, THUS REDUCING REACTION TO TEST FLASH STIMULUS
TENDS TO RAISE TEST-FLASH THRESHOLD
RESULT: TEST-FLASH THRESHOLD IS RAISED

Figure 3

ulus and the onset of the test flash will be referred to as the "conditioning interval."

I would like to turn now to a theoretical kind of schematization (Fig. 3) which summarizes why we thought, before the experiment began, that we might be able to drive the threshold down by light-adapting the eye. This represents the hypothetical effect of increasing the luminance of the pre-adapting stimulus.

The first thing which should happen, if the pre-adapting stimulus luminance is increased, is a decrease in photochemical availability—a desensitization of the eye for this reason. This should be true even three tenths of a second after the light is turned off. Some dark-adaptation has occurred; but, obviously, not nearly enough to get the eye back very far away from its light-adapted level. This, in turn, leads to a decrease of receptor sensitivity. This is fairly straightforward and, I guess, not too controversial up to this point.

Now we see that there are two sequences which should occur as a result of this decreased receptor sensitivity. Through the upper route, we would predict a decreased burst of "on" activity due to the onset of the conditioning stimulus, assuming that the conditioning stimulus is left at all times at the same level throughout the experiment. In other words, if you have a stimulus (the conditioning stimulus in this case) *which is at a given level* and, in one case, the eye has been light-adapted and, in the other case, the eye has been dark-adapted, there is ample physiological evidence to show that the burst of on-responses will be less in the case of the light-adapted eye. This correlates with the perception of brightness which will also be less for the conditioning stimulus in the case of the light-adapted eye.

This decreased burst of activity would mean, in terms of the kind of ideas I advanced a moment ago, a decrease in masking, somewhere in the visual system, produced by conditioning-stimulus onset. The consequence of this would be a *decreased* threshold to the test-flash. On the other hand, we have to remember that the threshold of the test flash is being determined in an eye which is being deprived of photochemical sensitivity by a more direct route because, after all, the increase in the luminance of the pre-adapting stimulus will also decrease the sensitivity of the receptors to the test flash, in the same way it did to the conditioning-stimulus onset. This consideration would lead to the more usual prediction of an *elevated* threshold to the test flash. The upshot of it all is that the threshold could go in either direction; and, as you will see, it actually goes in one direction first, then reverses itself and goes in the other.

To get down to the details for a moment, the variables in the experiment are these: The pre-adapting stimulus luminance was varied over a range of eight steps, from .00015 to 3000 mL. The conditioning interval was varied from −0.2 sec. +.03 sec. This means that, at one extreme, the conditioning stimulus came on 0.2 of a second before the conditioning-test stimulus; in the other case, 0.3 of a second afterward. The other variable is the luminance of the test flash, which is the dependent variable. This is the thing which is being adjusted until the subject can just barely see it. Held constant is the pre-adapting stimulus exposure, (in the sense that it is at least ten minutes or more, so that the subject has become completely adapted to it, and no further changes would ensue if it were left on any longer) the conditioning-stimulus luminance, which is 38 mL, and the dark interval (the time between offset of the pre-adapting stimulus and onset of the conditioning stimulus) which is 0.28 sec.

The results of part of the experiment are shown in Fig. 4. The thresholds obtained under the "on" condition are given here by the open circles in the upper curve; the thresholds obtained under the "off" condition are given by the solid circles in the lower curve. For the "off" condition (which

was a simple one, as you recall) the pre-adapting stimulus was turned off, and the test flash was subsequently delivered. The thresholds behave in a sensible way. They go up as a function of an increase in pre-adapting luminance. On the other hand, for the "on" condition, where we are complicating the situation, we notice that the threshold (as soon as it is first affected at all by a change in pre-adapting luminance) begins to drop and, as a matter of fact, drops nearly a full log unit. Finally, we get to a minimum, the curve reverses itself and starts to rise upward again.

Notice that the difference between these two curves is getting progressively smaller as the pre-adapting luminance is increased. This is taken to mean that the effect of the conditioning stimulus (which is the only difference between the two conditions) is becoming progressively less as the pre-adapting luminance is raised. As you will see, it is the on-response to the conditioning stimulus which we are concerned with here.

These results for the "on" condition verify the conclusion I gave you a moment ago about the threshold going down; also, they reveal some rather peculiar identities. For this situation, a pre-adapting luminance of zero produces the same threshold as for 2300 mL; other similar identities are found (for example, 575 and 10). In other words, you can cut across this curve horizontally at any point you want to, and find such identities. This is an unusual way for thresholds to behave as a function of a single variable which has something to do with intensity.

What we would like to do now would be to determine the masking effect of the conditioning stimulus (along the upper route on the theoretical diagram in Fig. 3) by trying to get rid, somehow, of the effects which we know are exerted directly upon the threshold of the test flash, because of the reduced photochemically-based sensitivity of the eye. This is the way the argument goes from this point: For the "off" condition, we can get an absolute cone threshold by looking to the condition where this pre-adapting luminance is zero. In this case, we are simply presenting a single test flash to the eye. The threshold here turns out to be on the order of .007 mL. This is indicated by the line labelled B_o.

As we raise the luminance of the pre-adapting stimulus to 30 mL, the threshold for the "off" condition (the single flash following the extinction of the pre-adapting stimulus) has been driven up to about .08 mL.

If we can assume—and I think this is an important and perhaps controversial assumption—that the threshold under the "off" condition is a reliable index of photochemical availability alone (not contaminated by any masking) we can say that the threshold is thus increased by a factor of twelve; and the eye is now deprived of 11/12 of its optimal sensitivity because of this photochemical bleaching. Would it not be true, if we could evaluate the effect of masking produced by the conditioning stimulus, but with an eye which somehow had not lost the photochemical availability caused by the pre-adapting stimulus, that the threshold would have dropped even more than it did? (We got a threshold drop, despite a twelve-fold decrease in photochemically-based sensitivity).

So the remainder of the argument is simply to say that the threshold would have gone twelve times lower than it actually did, provided we were able to evaluate the masking effect without using an eye which for photochemical reasons had been desensitized. If you work through the mathematics of this, you find out that you can calculate the middle curve (labelled "B_m") simply by taking the difference between the log threshold for the "on" and "off" conditions and adding it to the log absolute threshold value. This curve is taken to represent the best estimate we can get of what the threshold would have been as a result of the reduction of masking alone, without the loss of photochemically determined sensitivity. Thus, we label the curve

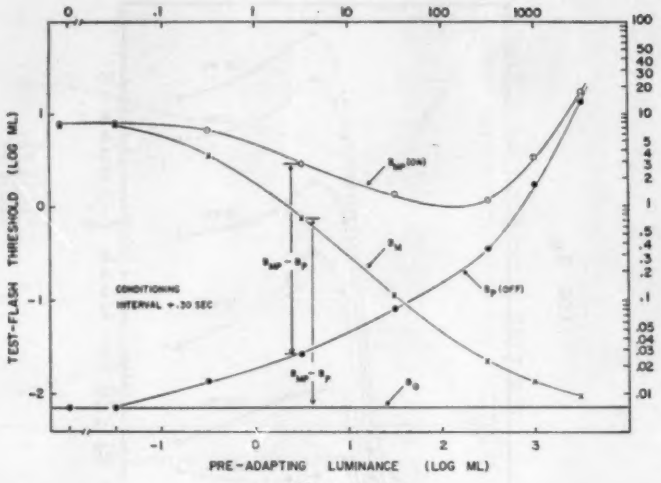

Figure 4

"B_m," and suggest that this may represent a way of getting at the amount of masking produced by the conditioning stimulus; and we see that it does drop in a very sensible way as pre-adapting luminance is increased. This kind of analysis indicates that the masking is very high if the eye has been previously adapted at a zero level; in fact that all of the threshold elevation is the result of masking; and that, as we go toward the other extreme, we are approaching a condition where all of the threshold elevation will be a result of photochemical bleaching alone. The point where we had to stop the experiment, because we did not want to injure ourselves permanently, was up at about 3000 mL, and here we see that the difference between threshold for the "off" and "on" conditions is very small. Interestingly enough, associated with this condition is the fact that the conditioning stimulus, still "plugging away" at 38 millilamberts, is scarcely visible by the subject. To put it simply, he is so light-adapted by this time that he can just barely see the 38 mL flash which, when he was dark-adapted, looked very bright indeed.

The curves of Fig. 4 were all taken for a conditioning interval three-tenths of a second positive. It is possible to derive a whole set of these curves for the other conditioning intervals we used. If you do this, then plot the data in a different way, with the conditioning interval along the abscissa, you get the family of curves which you see in Fig. 5. Here we see something which looks as though it may be a very reasonable picture of "on responses" in the visual system, where we note that, for the condition of zero pre-adaptation, the curve comes up quickly and reaches a high peak; then as pre-adapting luminance is increased these "responses" get smaller and smaller until, finally, when we get up to the highest levels of pre-adaptation, the change produced by the conditioning stimulus is very small indeed.

I would like to make one further observation about the data as plotted this way. Ultimately, if you expose the subject to the conditioning stimulus for a long enough time—(we only had it on for a half-second)—that is, if you kept him looking at it for, let us say, fifteen or thirty minutes, to be perfectly safe about it, eventually all of these curves are going to reach the same point. The reason for this is that the subject will become completely adapted to the conditioning stimulus; and the pre-adapting stimulus, which was turned off long ago will have lost its effect. This final level actually would be where the arrow is placed

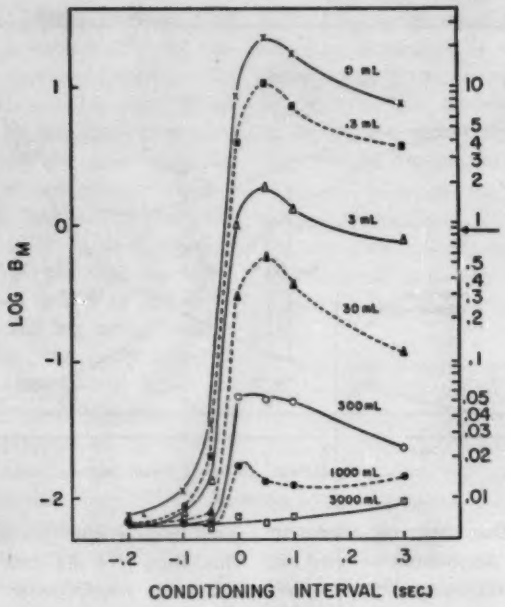

Figure 5

on the right-hand side of the graph. We see that the curves for the highest levels pre-adapting luminance have indeed begun to rise during the time the conditioning stimulus is still present. I will take this to be evidence of threshold change produced by photochemical factors, but notice that the threshold is going down in the case of the 30 mL curve, for example, in spite of the fact that, ultimately, it is going to have to rise and reach a higher level.

Having gotten to this point, a possible objection to this experiment occurred to us and to others; and that was this: This whole analysis is based on the assumption that you can, in fact, evaluate the sensitivity of the eye from a photochemical standpoint. In other words, it has been assumed that you can estimate the photochemical availability (sensitivity based upon photochemical availability alone) with a flash of light which is turned on shortly after the extension of the pre-adapting stimulus. "What about 'off' effects?" people have asked. If there are off effects, there would be some masking also produced by the pre-adapting system going off. For this reason, we decided to do an auxiliary experiment, a kind of control, using only one of the conditioning intervals, 0.05 of a second positive, to see whether we could get the same kind of results if we left the pre-adapting stimulus on throughout the entire cycle. So the pre-adapting stimulus is no longer a pre-adapting stimulus; it is simply an adapting stimulus or background stimulus, upon which the test-flash sequence is superimposed; otherwise, the experiment is exactly as it was before.

The results of this experiment are shown on the Fig. 6. We have plotted here, first of all, the results for the "on" and "off" conditions of the first experiment, (where the pre-adapting stimulus was turned off) and we see the same kind of curves as in Fig. 4, except that these are for a different conditioning interval. (This does not seem to be a crucial variable, as far as the main features of the functions are concerned.)

The actual experimental data, plotted as they should be for the "off" condition (the new experiment where the adapting stim-

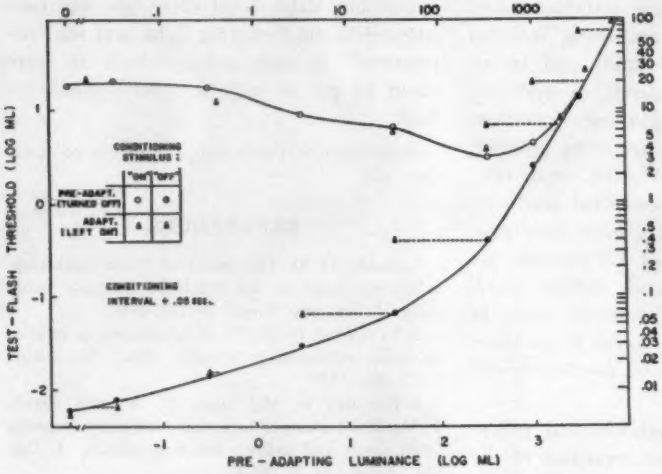

Figure 6

ulus is left on), are shown as the solid triangles. What happens is the sort of thing I think you might expect. An adapting stimulus which is of the same luminance as it was before will raise the threshold more, now that the adapting stimulus is still present, than when we allowed 0.28 sec. dark-adaptation. So the difference between the filled circles and solid triangles really represents the dark-adaptation which took place during the dark interval in the first experiment.

The question I would now like to pose is this: if we were to adjust the luminance of the *adapting* stimulus in the new experiment so that it produces the same effect, under the "off" condition, as the *pre-adapting* stimulus did in the original experiment, would we then continue to get the same result for the "on" condition? In other words, is a pre-adapting stimulus which is turned off equivalent in all respects to one which is left on, except for the sensitivity loss resulting from photochemical considerations, during this initial period of dark-adaptation?

This question can be answered rather readily, simply by taking each of the points on the new curve and coming on across horizontally until we strike the function which represents the curve where the pre-adapting light was turned off. This yields the level of pre-adaptation in the original experiment, which we needed to produce the same effect which we are now getting. It just happens, for example, that 30 mL pre-adaptation produces the same "off" threshold as 3 mL prevailing adaptation. We now plot the results for the "on" condition of the new experiment, at each adjusted pre-adapting luminance value, to see whether the effects for the "on" condition are the same. We find that, within the limits of the experimental variability, they do appear to be very close. I would not want to say they are exactly the same, because this would involve proving the null hypothesis.

These, then, are the data which provide the experimental evidence upon which the conclusions mentioned earlier have been based.

What, if anything, is the importance of all this?

Sometimes, in talking to my visual friends around the country, I get the impression that some of them consider visual psychophysics passé. It certainly is true (as we have heard here the last two days) that each day more and more ingenious techniques are being developed in an effort to further our knowledge of the visual process. Eyes are being stimulated by electricity; light is being reflected from them at

the cornea various optical interfaces, and the fundus; electrodes are being inserted into the system—of all kinds and in all conceivable places; thousands of eyes are being ground up for the purpose of extracting photopigments; holes are being punched in the back of eyes so see what comes out; and so on. These are wonderful activities. But, psychophysical data always have provided, and, I think always will provide, the basic behavioral framework within which these other bits of information must fit and make sense. This is "point 1" in favor of a continued emphasis on psychophysical data.

"Point 2" is that psychophysical procedures are among the most important of the clinical tools. From the prescription of eyeglasses to the diagnosis of glaucoma, the so-called "subjective" methods are indispensable. They have not always been used properly, nor are they beyond improvement.

I would like to close by indicating where we expect to turn next in our studies. I would also like to mention that we are very happy to see others, like Dr. Battersby, do similar types of experiments in other laboratories.

(1) *The investigation of color vision.* We have already found[10] that the selectivity of chromatic adaptation is much greater during the period where masking is important than it is under steady-state conditions. Thus, we have been able to obtain spectral sensitivity curves having very unusual shapes, which may help to reveal the underlying processes of color vision.

(2) As an outgrowth of this, it should be possible to build a simple device, for clinical use, which may possibly prove to be a superior and simpler test of color vision than some now in use. There is only one way to find out, and that it to try it.

(3) We plan to take a new look at flicker with this method. As I see it, cff depends upon a balance of the two factors that have been stressed in this paper—photochemical availability, which reaches a fluctuating, but reasonably stable level after one has been adapted to the flickering light, and the "on-response" to each pulse—which in turn could be got at with a superimposed test flash.

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